Sustainable plant-derived coagulating agents in a heterogeneous platform for front-end water purification

by

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EXECUTIVE SUMMARY

Chemical coagulants have been associated with having adverse economic, health and environmental impacts. Many rural communities do not have access to specialised coagulating agents and infrastructure for water treatment and resort to consuming water from unprotected sources. It is therefore of utmost importance to develop a simplistic, low-cost, easily accessible and environmentally friendly technique of water treatment that can be applied in rural communities. Although homogeneous coagulating agents, derived from plant species, have been investigated for this purpose, their use results in increased organic matter in the treated water, and the production of flocs with poor settling characteristics. Therefore, homogeneous coagulant proteins, from *Glycine max, Cicer arietinum, Vigna unguiculata and Vigna mungo,* were loaded onto silica substrates to produce a heterogeneous coagulant product, which would act as a binding site for suspended inorganic and organic matter and lead to improved floc settling characteristics. Experimentation was conducted to compare the efficiency of heterogeneous coagulants derived from biomass sources in turbidity removal and thereafter to establish the optimum conditions for front-end water treatment. The active components were extracted from the seeds of each plant species via aqueous extraction (using distilled water as the extraction medium) and salt-extraction (using sodium chloride salt solutions as the extraction medium). The salting-in process, which involved increasing the salt concentration of the extraction medium, was employed to improve protein extraction into solution. The optimum salting-in concentration for extraction was 0.4M, 0.8M, 0.5M and 0.3M for *Vigna unguiculata, Cicer arietinum, Glycine max* and *Vigna mungo* respectively. In order to improve protein loading, the salt concentration was raised to facilitate the salting-out process. The optimum salting-out concentration was 0.4M, 0.825M, 0.6M and 0.75M for *Vigna unguiculata, Cicer arietinum, Glycine max* and *Vigna mungo* respectively. In order to establish the optimum dose of the heterogeneous coagulating agent, turbidity removal experiments were conducted by means of a jar test. This involved the preparation of synthetic turbid water samples (at an initial turbidity of 200 NTU ± 0.5 NTU) and varying the dose of the aqueous extracted heterogeneous coagulant and the salt-extracted heterogeneous coagulant. The results of the jar test revealed that the optimum dose and corresponding average turbidity removal of aqueous extracted heterogeneous coagulant derived from *Vigna unguiculata, Cicer arietinum, Glycine max* and *Vigna mungo* was 15g/l and 80%, 15g/l and 78.2%, 20g/l and 79.1% and 10g/l and 82.3% respectively. The optimum dose and corresponding average turbidity removal of salt-extracted heterogeneous coagulating agent was 5g/l and 89.6%, 10g/l and 80.4%, 10g/l and 87.4% and 5g/l and 89.6% for *Vigna unguiculata, Cicer arietinum, Glycine max* and *Vigna mungo* respectively. This proved that higher turbidity removal efficiencies can be achieved at lower salt-extracted coagulant doses. From the study, the heterogeneous coagulant derived from *Vigna mungo* was deemed the most effective due to its high turbidity removal efficiency at low doses and low salt concentration requirements for extraction and loading. In addition, its drought-resistance, versatility, short maturity period, simple harvesting requirements, non-toxicity and biodegradability makes this plant species an ideal candidate for front-end water treatment.

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NOMENCLATURE

Symbols	Description
AR	Analytic reagent
C	Concentration [M]
CEC	Crop estimates committee
CP	Chemically pure
DAFF	Dissolved air flotation and filtration
HPLC	High-performance (or high pressure) liquid chromatography
JTU	Jackson turbidity unit
m	Mass [g]
MM	Molecular mass [g/mol]
MOCP	*Moringa oleifera* cationic proteins
n	Number of mols [mol]
NOM	Natural organic matter
NTU	Nephelometric turbidity unit
R^2	Correlation coefficient
SANS	South African National Standard
S_{xx}	Sum of squares of x's
S_{yy}	Sum of squares of y's
S_{xy}	Sum of products of x and y
T_i	Initial turbidity [NTU]
T_f	Final turbidity [NTU]
TOC	Total organic carbon
TSS	Total suspended solids
V	Volume [ml]
$\bar{x}$	Mean of x values
x_i	i^{th} x value in the data set
$\bar{y}$	Mean of y values
y_i	i^{th} y value in the data set
σ	Standard deviation

CHAPTER 1: INTRODUCTION

1.1. Background

Although almost 71% of the Earth is covered in water, only 2.5% of the Earth's water is freshwater (most of which are trapped in glaciers and snowfields). Thus, approximately 0.007% of the Earth's water is available for human usage in purposes such as consumption. Further, much of this water is inaccessible and/or unsafe for consumption without treatment (National Geographic, 2020).

It is estimated that a staggering 3 in 10 people worldwide lack easy access to safe water (WHO, 2017). In addition, every year, 361 000 children under 5 years old die as a result of diarrhoea. Although South Africa boasts one of the cleanest water supply systems in the world, the lack of sanitation and access to water in certain areas have resulted in the contraction of water-borne diseases still being a prominent threat.

It is anticipated that the global demand for freshwater will exceed its supply by 2050 (Yani, et al., 2019). Exponential population growth as well as social economic development and its associated activities have placed immense strain on fresh water supply, through excessive consumption and plethoric pollution. In addition, increased heatwaves, sporadic rainfall and drought, due to climate change, has plunged many regions into a state of crisis.

1.2. Rationale

Pre-treatment of water prior to consumption is a vital process to reduce contamination, and thereby prevent transmission of water-borne diseases. Conventionally, water-treatment consists of processes such as coagulation and flocculation, sedimentation, filtering and disinfection (Centers for Disease Control and Prevention, 2015). Decentralised water treatment systems are preferred, especially in developing countries, as they promote sustainable development, are practical, simple and low-cost since they are generally small-scale operations. The decentralised treatment system most commonly involves the coagulation process as it offers the benefit of being effective, simple and easily applicable to both rural and urban areas (Yani, et al., 2019).

Although specialised chemical reagents for coagulation in water treatment exist, high cost and availability in developing areas poses a major challenge. More cost-effective traditional reagents, such as aluminium sulphate, are effective, however, at high concentrations, they give rise to turbidity, reduce disinfection efficiency and have been linked to neuropathological diseases such as Alzheimer's disease (Srinivasan, et al., 1999). It has therefore become a necessity to develop a low-cost, safe, readily available, and environmentally friendly treatment system involving plant-based natural materials.

Plant material from *Moringa Oleifera* has been widely studied to serve the purpose of water treatment. Soluble proteins extracted from the seeds of this plant can be used as a coagulant for turbidity reduction (Choudhary & Neogi, 2017). The extracted proteins from this plant are also found to have excellent antimicrobial properties and are biodegradable (Choudhary & Neogi, 2017).

Moringa Oleifera has showcased excellent coagulation performance in literature, however there are limitations to its use in water treatment such as the requirement of a high coagulant dose which results in an increase in the total organic carbon(TOC) in the water body, the need for a long sedimentation time to achieve suitable turbidity removal efficiencies, the lack of complete pathogen removal, and the relatively low turbidity removal performance in low to medium turbidities (Awad, et al., 2013). Studies have also shown that roasted dry *Moringa oleifera* seeds contain mutagenic, diuretic and genotoxic compounds, however toxicological studies, involving the use of *Moringa oleifera* for water treatment, requires further investigation. In addition, due to its coagulation properties, sufficient purification of *Moringa oleifera* components is required prior to its use in water treatment and the high costs associated with the purification may be higher than the overall cost of conventional coagulants (such as aluminium sulphate) (Ndabigengesere & Narasiah, 1998).

This study was designed to investigate the performance of other plant species in turbidity removal, to overcome the limitations associated with the use of *Moringa oleifera*. The further benefit of the exploration and application of other plant species, in lieu of *Moringa oleifera,* as a potential coagulant, is that communities can consider a greater variety of plant sources for treatment should there be a lack of availability of commonly used plant coagulants such as *Moringa oleifera*.

Literature surrounding the topic of the use of plant-based natural coagulants has been majorly focused on aqueous extraction and application of homogeneous coagulants. However, studies have shown that major concerns arise when plant-based homogeneous coagulating agents, such as *Moringa oleifera*, are utilised in water treatment. Firstly, high doses of homogeneous coagulant are generally required to achieve satisfactory turbidity removal. Secondly, due their organic nature, the use of crude proteins, especially in high doses, may promote microorganism growth and changes in colour, taste and odour, if stored for long periods of time. In addition, the organic matter in the treated water may consume chlorine in the disinfection stage of treatment and can lead to the formation of disinfection by-products such as trihalomethanes (which have been associated with negative health risks). Thirdly, flocs from induced coagulation and agglomeration using homogeneous coagulants have poor settling characteristics and would require large settling tanks to remove the sludge from the treated water (Ndabigengesere & Narasiah, 1998).

Since most studies in literature involve the use of plant-derived homogeneous coagulants, there is a lack of extensive research into the use of heterogeneous plant-based coagulants, particularly focused on the optimum conditions for extraction, loading and turbidity removal. Investigation into a heterogeneous platform for the development of a plant-based coagulant was a worthwhile study due to the limitations associated with the homogeneous coagulant. Studies have proven that these limitations can be avoided through the use of heterogeneous coagulants, which are prepared via the immobilisation of plant-derived proteins onto a substrate. Heterogeneous plant-derived coagulants offer several benefits such as having the ability to retain coagulation properties and allowing for organic matter to be easily rinsed away (Nisha, et al., 2017). In addition, *Moringa oleifera*-derived heterogeneous coagulants studied by Nisha, et al. (2017) were found to be effective in removing suspended particles, and lowering the total dissolved solids, total hardness, chloride concentration and electrical conductivity. The turbidity removal efficiency and pathogen removal capabilities of the heterogeneous coagulant, produced from *Moringa oleifera,* was also evidenced in a study by Jerri, et al. (2012). In addition, Nordmark, et al. (2018) found that the heterogeneous coagulant, prepared by the immobilisation of *Moringa oleifera* cationic proteins onto silica, would be stable upon reuse.Thus, current studies in literature involving heterogeneous coagulants derived from *Moringa oleifera* have substantiated the investigation into heterogeneous coagulants derived from plant species other than *Moringa oleifera.*

The focus of this study was to compare the turbidity removal performance of heterogeneous coagulating agents derived from four different plant species. Due to the lack of literature regarding the optimum conditions for maximised performance of heterogeneous coagulants produced from plant species other than *Moringa oleifera,* this study served to provide insight into the turbidity removal performance of heterogeneous coagulating agents derived from *Glycine max, Cicer arietinum, Vigna unguiculata, and Vigna mungo*, under optimal conditions.

Two methods of protein extraction, aqueous and salt extraction, were investigated in this study, to recover the active components from the raw plant material. The former was chosen for its simplicity and low cost. However, studies have shown that coagulating protein extractability from biomass sources are generally low, therefore large quantities of raw material would be required for large-scale operations. Further, only a small fraction of proteins extracted from biomass sources, via aqueous extraction, are active for coagulation. To overcome these limitations, salt extraction(salting-in) was employed as a means of improving the extractability of active coagulating proteins. Salt extraction was a viable alternative as studies have shown that salts, such as sodium chloride, are capable of increasing protein solubility and thereby allowing for greater amounts of protein to extracted (Gordon & Barbut, 1992). However, the increased protein solubility was anticipated to pose difficulties upon loading, therefore salting-out was also investigated to allow for proteins to precipitate out of solution prior to being adsorbed onto a substrate. The optimum salting-in and salting-out concentration was established to maximise the extractability and loading of each plant species.

Jar tests were used to evaluate the performance of the heterogeneous coagulating agents derived from the plant species under investigation. The coagulant dose added to each sample of turbid water, needs to be carefully controlled to prevent the formation of weak and small flocs, due to underdosing, or charge reversal, due to overdosing (Yani, et al., 2019). Therefore, since floc formation in water treatment is greatly influenced by the dose of coagulant added to a sample of turbid water, the optimum dose of each heterogeneous coagulant product was investigated to ensure maximised turbidity removal performance of each plant species.

1.3. Aims and objectives

1.3.1. Aim

With the advent of climate change, widespread environmental degradation, and rapid depletion of the Earth's natural resources, an investigation into a natural coagulant for water treatment would be a worthwhile endeavour that would align with global sustainable development initiatives. The aim of this study is to develop and optimise a cost-effective, natural and biodegradable heterogeneous coagulating agent, using different plant-derived proteins (*Glycine max, Cicer arietinum, Vigna unguiculata, and Vigna mungo*), that can be utilised more effectively in the treatment of turbid water, primarily in areas lacking improved water sources. Further, this study will allow for the provision of a renewable and natural resource for water treatment that could potentially reduce the dependency on inorganic, and potentially harmful, treatment chemicals, such as aluminium sulphate.

1.3.2. Objectives

- To undertake a comprehensive literature review which will involve extensive research into different biomass sources, various methodologies for efficient production of an optimal natural coagulating agent, and the coagulation mechanisms involved in treatment.
- To undergo experimentation for the development and testing of heterogeneous coagulating agents derived from active components of plant materials. Experimentation would involve aqueous extraction (using distilled water) of coagulating proteins from four plant species (*Glycine max, Cicer arietinum, Vigna unguiculata, and Vigna mungo)* for loading onto silica substrates for independent testing of coagulation activity.
- To optimise the protein extraction process using sodium chloride salt solutions in the salting-in and salting-out method to promote loading of the proteins onto silica substrates in the next stage.
- To establish the optimum conditions for extraction, loading and turbidity removal of each plant species by determining the salting-in concentration that maximises protein extraction, the salting-out concentration that maximises protein loading and the optimum dosage of heterogeneous coagulating agent that maximises turbidity removal.

1.4. Thesis outline

This thesis comprises of 7 chapters. Chapter 1 provides a brief background and rationale for this study. In addition, chapter 1 outlines key aims and objectives that this study intends to achieve. Chapter 2 presents a comprehensive review of the literature surrounding the subject matter under investigation. This chapter provides insight into turbidity and water treatment methodologies that are conventionally applied to produce clean and safe drinking water. In chapter 2, emphasis is placed on the coagulation process since the focus of this study is front-end water treatment. Details of various inorganic and organic coagulants are also provided in this chapter. Since this study serves to investigate the use of natural plant-derived coagulants, the methodologies for the production of heterogeneous coagulants from the raw material stage, are also explored.

Chapter 3 provides an outline of the experimental design, with emphasis on the procedure for extraction, loading and dose optimisation. This chapter also contains descriptions of the material and equipment used for the experimental portion of this study. Further, chapter 3 includes a detailed experimental procedure for the production and testing of optimised heterogeneous coagulants.

Chapter 4 presents the experimental results of this study as well as comparative results from literature sources. Chapter 5 provides an in-depth discussion of the experimental findings in this study, with emphasis placed on the results from extraction, loading and turbidity removal testing. A section detailing how the experimental methodology can be adapted for use in rural communities is also found in chapter 5. In addition, insight into the environmental, social and economic impacts of the production and use of the heterogeneous coagulants investigated in this study is provided in chapter 5.

Chapter 6 provides the conclusions derived from this study and finally chapter 7 includes recommendations that would be worthwhile investigating in future studies of this nature.

CHAPTER 2: LITERATURE REVIEW

2.1. Current situation

With the advent of climate change, exponential population growth, and demographic changes, additional strain is placed on water sources and supply systems. It is a basic human right to have easy access to safe and affordable drinking water. However, in 2017, it was noted that approximately 2.2 billion people globally did not have access to safely managed drinking-water services (clean, on-premises water sources). Of the 2.2 billion people, 144 million relied on untreated water sources such as rivers, ponds and streams (WHO, 2017). Unsafe, contaminated drinking water and poor sanitation poses a high risk for the contraction of diseases such as cholera and diarrhoea. In addition to the staggering 1 million yearly death rate due to water, sanitation and hygiene-related diseases, many people experience physical injury from carrying heavy water containers for long distances (WHO, 2019). Poor families are placed under further financial strain due to the exorbitant healthcare expenses they incur treating these ailments.

Women and children bear the brunt of the water crisis. It was reported that women and girls globally spend 200 million man-hours a day collecting water. The copious amounts of time spent carrying out this task prevent many from performing traditional roles at home such as attending school and going to work (Graham, et al., 2016). Further, due to the health risks associated with poor sanitation and lack of water, many children choose not to attend school. Without an adequate education or the ability to financially provide for their families, this then leads to people, particularly women, becoming trapped in a vicious cycle of poverty. Overall, it is anticipated that every $1 invested into water and sanitation would yield a $4 financial return due to increased productivity, reduced healthcare expenses and lower death rates (WHO, 2012). These alarming figures indicate that it is crucial to develop a swift resolution to the low clean water supply currently available to an increasingly demanding society.

2.2. Turbidity

2.2.1. Description

Suspended solids usually constitute organic and inorganic matter. Organic material results from living organisms such as leaves, seeds, moss, algae and animal matter. Inorganic material usually constitutes silt and clay, resulting from physical causes, and calcium, magnesium, iron and manganese, resulting from chemical causes. Some suspended solids, that are generally heavier than others, tend to sink to the bottom of a stagnant water body. Suspended solids can range from large sand and gravel particles to fine clay and silt particles. The solids that do not settle however, due to their small size and low density, are known as colloidal or non-settleable solids (Fondriest Environmental, Inc., 2014).

The concentration of natural organic matter (NOM) in a body of water varies based on water chemistry, pH, temperature, seasonal changes and the different types of biological processes occurring. The presence of NOM in water sources affects the colour, taste and odour of water and consequently reduces the water quality (Ncibi, et al., 2017). Upon the reduction of NOM using chlorine (a process commonly used in conventional water disinfection processes), disinfection by-products (such as trihalomethanes and haloacetic acids) are produced, which are potentially harmful to human health (Bond, et al., 2012; Goslan, et al., 2017). Thus, the presence of such a variety of organic matter, amongst other substances, has reinforced the requirement for a specialised, versatile and efficient water treatment system, especially if the end-use is consumption (Ncibi, et al., 2017).

Turbidity is a measure of the reduction in clarity of a fluid as a result of the presence of organic and/or inorganic particles that may be suspended or dissolved (Department of Environmental Affairs, 2018). Consequently, turbid water tends to appear cloudy, hazy, muddy or murky. Turbidity measurements thus serve as a means of establishing water quality based on the transparency and the amount of total suspended solids (TSS) present in the water. Suspended particles absorb or scatter downwelling light and serve as absorption and desorption sites for different substances such as various types of organic matter (Grobbelaar, 2009). Turbidity is not a direct measure of the quantity of solids present in water but rather turbidity measurement establishes the relative water clarity by determining the amount of light scattered in the water body. This concept is based on the relationship between the amount of solids and the amount of light scattered, where more solids tend to scatter more light (Fondriest Environmental, Inc., 2014). Turbidity is usually reported in nephelometric turbidity units (NTUs). The primary standard for turbidity is the polymer formazin. This polymer was matched to a mass of kaolin clay, where 1 NTU approximately equals 1mg/l kaolin.

2.2.2. Sources of turbidity

The main sources of turbidity are natural and human-induced sources. Natural sources include soil erosion, run-off, residual decay of plant and animal matter, resuspension of material deposits by bottom-feeders (such as carp) and excessive algal growth. It must be noted that increased volume and flow within a water body, due to increased rainfall, tide, wind and other sources, escalates soil erosion, runoff and resuspension of material. Human contributions to turbidity in water bodies include construction, mining, fires, dredging and tree logging which can induce soil erosion, result in waste discharge and urban run-off. Further, direct contamination of water with bacteria and other pathogens by sewage plant discharge can heighten turbidity levels and pose health risks. Indirect sources are found in the agricultural sector, where animal waste, and the use of fertilisers and pesticides for farming activities result in these nutrients and chemicals being washed into water bodies during heavy rainfall; consequently, algal bloom is encouraged, due to the increased nutrient content, which further increases turbidity levels.

2.2.3. Analytical measurement of turbidity

2.2.3.1 Jackson candle turbidity meter

The Jackson candle turbidity meter (shown in Figure 1) was the first practical instrument used in turbidity measurement. It consists of a candle, a calibrated flat-bottomed glass tube and a support to align the candle and tube. The water sample is added into the glass tube, and held over a lit candle, until the viewer can no longer see the candle flame. When the flame can no longer be seen, it means that the intensity of the scattered light equals to the transmitted light. The depth at which this occurs is recorded and compared to a reference ppm-silica scale and the turbidity is finally realised in Jackson turbidity units (JTU) (Shauri, 2006; Johnson, et al., 2007).

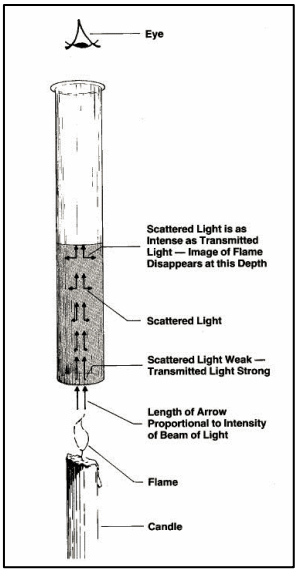


Figure 1: Jackson candle turbidimeter schematic (Johnson, et al., 2007)

The Jackson candle turbidimeter, although a simple and low-cost method of turbidity measurement, has a limited application and cannot measure turbidity levels below 25 turbidity units. It is therefore better suited for the measurement of highly turbid waters (Johnson, et al., 2007).

2.2.3.2. Secchi disk

The most simple and inexpensive method of turbidity measurement involves the use of a Secchi disk. The Secchi disk usually consists of a weighted circular plate, that is approximately 20cm in diameter and has alternative black and white quadrants. The disk is attached at its center to a line that ensures that the disk hangs horizontally. The disk is lowered into a body of the water until it is no longer visible (Kar, 2016). The Secchi disk operates on the principles of light absorption within a water body. Water clarity or lack thereof (turbidity) can be established by determining the depth that sunlight penetrates water (the photic zone depth). The greater the depth of the photic zone, the clearer the water (Fondriest Environmental, Inc., 2014). Thus, by determining the depth at which the Secchi disk is no longer visible, an indication of the water turbidity can be attained (as shown in Figure 2).

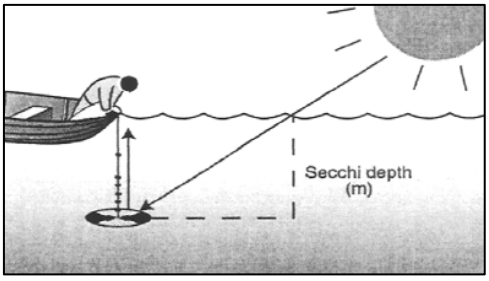


Figure 2: Methods used to determine Secchi depth (Short & Coles, 2001)

The Secchi disk offers many advantages such as its ease of operation, its quick and cost effectiveness in data collection, as well as its wide range of applicability to different habitats. However, this method tends to be a slower means of measurement as opposed to electronic equipment (such as the nephelometer) and there may be operator bias involved as it depends on eyesight accuracy.

2.2.3.3. Transparency tube (turbidity tube)

The transparency tube (shown in Figures 3 and 4) consists of a demarcated long cylindrical tube with a Secchi disk attached at the bottom. Similar to the Secchi disk, this instrument uses the relationship between visibility and turbidity. By pouring water into the cylindrical column and visually establishing the level at which the Secchi disk pattern can no longer be seen, one can correlate the height of water to the water turbidity. Although this method is not as accurate as an electronic means of measurement and cannot measure turbidity levels below 5 NTU, it is still vastly employed for its ease of use, portability and low cost (Myre & Shaw, 2006). This method is preferred over the Secchi disk when a smaller body of water or small sample of water is tested.

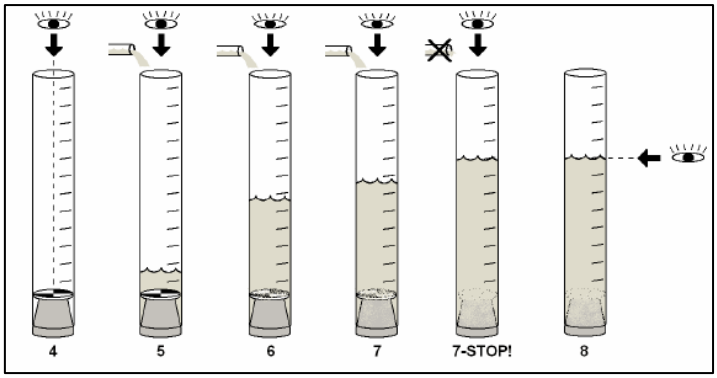


Figure 3: Method for use of a transparency tube (Myre & Shaw, 2006)

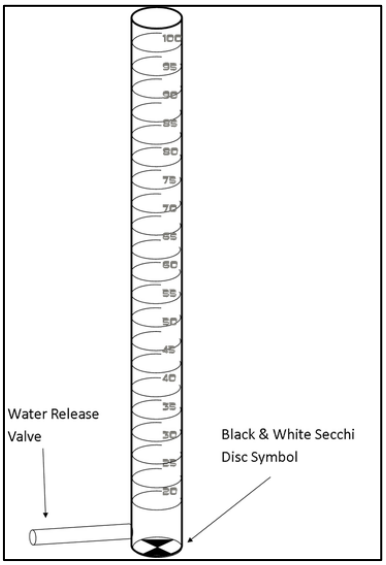


Figure 4: Transparency tube schematic (Fetter & Kyler, 2017)

2.2.3.4. Turbidimeter (nephelometer)

The turbidimeter or nephelometer is an electronic device that is able to provide highly precise and accurate turbidity readings. The nephelometer operates on the principle that states that colloidal particles in a water body tend to scatter light. By establishing the light scattering intensity, the relative quantity of colloidal particles can be gauged through turbidity. The nephelometer operates as follows; the device sensor directs a focused beam into a body of water, the light then becomes scattered by the particles present in the water, and finally the resulting light intensity from particle reflection is measured by the device photodetector, that is placed at a 90-degree angle to the initial light beam. The light intensity is then converted to a turbidity measurement. Figure 5 shows the optical geometry required for use of the nephelometer. The nephelometer is widely used in a variety of operations such as water quality testing, river monitoring, groundwater testing and wastewater treatment for its high accuracy, portability and ability to monitor low turbidity. Although, this device has a myriad of advantages, it is expensive, easily damaged, and requires calibration and a power source (Myre & Shaw, 2006).

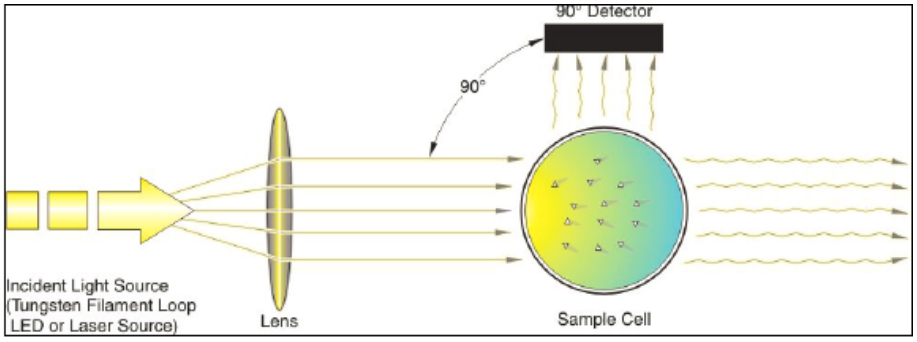


Figure 5: Optical geometry required for turbidity measurement using a nephelometer (Sadar, 2019)

2.2.4. Impacts of turbidity

2.2.4.1. Human impact

High levels of turbidity within water bodies, such as lakes and streams, can diminish its aesthetic appeal which may have severe repercussions on recreation and tourism in the area. Consequently, valuable income generated through these activities lessens. Further, negative economic impacts result due to the increased costs associated with water treatment, especially if the end-use is human consumption. Increased turbidity within a water body reduces navigability of ships and boats, which may hinder transportation; this consequently negatively impacts the economic sector. Turbidity has also been associated with the reduction in the capacity of water bodies such as rivers, lakes and dams. This then indicates that a reduced water supply will be available for human activities. Contaminants in water bodies, such as heavy metals and bacteria, may pose a serious health risk to humans, especially to those communities that do not employ proper water treatment techniques prior to use. Further, high turbidity may result in disinfection problems in water treatment systems. If suspended materials are not adequately filtered out, they may also pose health risks to humans. The impacts of different turbidity levels are summarised in Table 1.

Table 1: Effects of different turbidity levels on human health and aesthetics (Department of Water Affairs and Forestry, 1996)

Turbidity Range (NTU)	Effect on human health and aesthetics
0-1	Turbidity is not visible. There are no adverse health impacts and no adverse aesthetic effects
1-5	Turbidity is not visible. There is a small chance of adverse aesthetic and disease transmission
5-10	Turbidity is visible. There is some chance of transmission of disease by micro-organisms such as viruses and protozoan
>10	Turbidity is visible. Severe aesthetic effects on appearance, taste and odour. There is a chance of disease transmission at an epidemic level

2.2.4.2. Impact on aquatic life

High levels of turbidity affect water quality by increasing water temperatures and decreasing dissolved oxygen levels. This is due to the phenomenon that states that suspended particles absorb a lot of heat from solar radiation and transfer this heat to the surrounding water particles. In addition, stratification of water due to increased temperatures, may result in low oxygen content in the lower layers of a water body due activities such as cellular respiration. Thus, low dissolved oxygen content results in high aquatic organism death rates (Fondriest Environmental, Inc., 2014).

With an increase in turbidity, suspended particles within water bodies reduce the amount of sunlight reaching aquatic plants. The reduced light intensity then lessens or completely ceases photosynthesis processes. Consequently, plants are not able to generate food for themselves and die. Thus, they are unable to provide oxygen, through photosynthesis, which reduces the dissolved oxygen content of the water. The effect of this is two-fold, firstly since plants are not able to provide food for themselves, plant life rapidly diminishes, further, organisms that rely on plant life as a food source soon die as well. The death of various organisms within a water body then causes a spike in bacterial growth, to decompose these organisms. Secondly, with reduced photosynthesis (due to reduced plant life) and increased cellular respiration (due to increased bacterial growth), the dissolved oxygen content in the water decreases which leads to further death of microorganisms.

Pollutants, possibly as a result of human activities, can result in high levels of heavy metals and bacterial, nutrient and pesticide contaminants, which are highly toxic and detrimental to aquatic life. Freshwater fish are also negatively impacted by the presence of fine particles as it can directly kill them, reduce their growth rate and resistance to disease, prevent good development of fish eggs, change migration patterns, and reduce efficiency in fish catching (Minnesota Pollution Control Agency, 2006). Predator-prey interactions are also affected since with an increase in turbidity comes a reduction in light(which is necessary for hunting), therefore predation rates rapidly decline (Grobbelaar, 2009).

2.2.5. Turbidity removal

Conventional processes exist to successfully reduce turbidity levels. These processes generally involve coagulation, flocculation, sedimentation, filtration and disinfection. The reduction of turbidity is vital to ensure a safe and clean water supply. Once conventional processes are carried out it is necessary to quantify how much the turbidity has been reduced; this is accomplished by the following formula (Choudhary & Neogi, 2017):

$$Turbidity\ Removal = \frac{T_i - T_f}{T_i} \tag{2.1}$$

Where, T_i is the initial turbidity of the sample (NTU)

T_f is the final turbidity of the sample after treatment (NTU)

2.2.6. Turbidity standards

Table 2 indicates the acceptable standards for turbidity in water treatment. It is of utmost importance to refer to these guidelines to ensure that the end-product from water treatment is safe for use, especially if it is intended for consumption purposes.

Table 2: Turbidity standards in water treatment processes (WHO, 2017)

<table>
<tr><th colspan="2">Source or process Step</th><th>Turbidity standard</th></tr>
<tr><td colspan="2">Household water treatment and storage</td><td>Ideally <1 NTU, however if this is not possible, turbidity should be maintained <5 NTU. However, above 1 NTU, higher disinfection doses/contact times will be necessary.</td></tr>
<tr><td colspan="2">Aesthetic appeal</td><td>Ideally <1 NTU. Above 4 NTU, water bodies appear cloudy</td></tr>
<tr><td rowspan="5">Filtration</td><td>Conventional filtration</td><td rowspan="3">≤0.3 NTU but should not exceed 1 NTU</td></tr>
<tr><td>Dissolved air flotation and filtration (DAFF)</td></tr>
<tr><td>Direct filtration</td></tr>
<tr><td>Slow sand filtration</td><td rowspan="2">≤1 NTU but should not exceed 5 NTU</td></tr>
<tr><td>Diatomaceous earth</td></tr>
<tr><td>Membrane filtration</td><td><0.1 NTU</td></tr>
<tr><td rowspan="2">Disinfection</td><td>Well-run municipal supplies</td><td>Ideally <1 NTU. However, should be able to achieve turbidity levels below 0.5 NTU always, and average turbidities ≤0.2 NTU. However, above 1 NTU, higher disinfection doses/contact times will be necessary.</td></tr>
<tr><td>Small supplies</td><td>Turbidity should be maintained <5 NTU. However, above 1 NTU, higher disinfection doses/contact times will be necessary.</td></tr>
</table>

The South African National Standard (SANS) 241 provides drinking water specifications (such as pH, conductivity, turbidity, and total dissolved solids) which can be used to assess whether potable water is safe for consumption. Similar to the WHO standards shown in Table 2, SANS 241 states that turbidity levels greater than 5 NTU pose an aesthetic risk (which means that the water is aromatically, visually, and palatably unacceptable) and levels greater than 1 NTU pose an operational risk (which indicates that operational procedures that ensure that water parameters have met quality standards have failed). Further, SANS 241 indicates that the pH of drinking water at 25˚C should be 5-9.7 to prevent an operational risk (SABS, 2015).

2.3. Conventional water treatment methodologies

Numerous water treatment techniques exist however the conventional processes of coagulation, flocculation, sedimentation, filtration and disinfection (shown in Figure 6) are universally employed. In any water treatment system, it is cardinal to produce water of high quality. This implies that the end-goal of water treatment should be high purity water that is free of contaminants, and in accordance with water quality standards and guidelines.

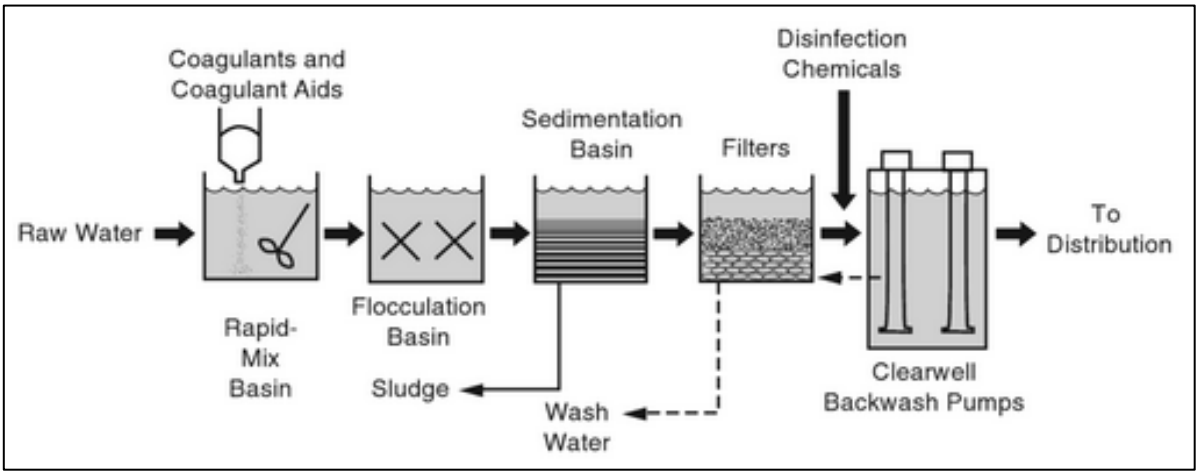


Figure 6: Conventional water treatment process (Pizzi, 2010)

2.3.1. Coagulation

Coagulation involves the addition of a coagulant to untreated water to enable it to react with impurities such as inorganic particles (like clays), pathogenic microbes and dissolved natural organic matter, in order to destabilise and neutralise electric charges in the particles (Tetteh & Rathilal, 2019). After the reaction occurs, the coagulant and impurities in combination are known as “floc”.

Coagulation can occur via four mechanisms: double layer compression, charge neutralisation, sweep coagulation and finally polymer bridging. Most colloidal particles found in water are negatively charged and can be regarded as stable due to electrical repulsion. When there is a high ionic concentration in the solution, compression in the volume of the double layer that encases colloidal particles occurs. This consequently reduces the repulsive forces of the particles, which makes them unstable and more prone to coagulation. This is known as double layer compression (Kristianto, 2017). Destabilisation of the colloidal impurities can be attained through the addition of either considerable quantities of salts or lower amounts of specialised cations that interact specifically with negatively charged colloids and neutralise their charge. Using specialised counterions is the most practical and efficient method of destabilisation as lower salt quantities are required. However, the optimum conditions for coagulation need to be applied, as excessive dosages can result in charge reversal and restabilisation of the colloids (Duan & Gregory, 2002).

The second mechanism is charge neutralisation. This mechanism occurs when coagulant polymers (which are opposite in charge to the particles in the water) are adsorbed onto particle surfaces and neutralise the charge of the colloidal particles. At optimum doses, a zeta potential of zero (neutral charge) ideally occurs, however this is practically difficult to attain therefore a slightly negative or positive charge typically occurs. The overall reduction in charge leads to a decrease in electrostatic repulsion among particles which promotes coagulation. For this purpose, coagulants with a high charge density and low molecular weight are preferred for enhanced performance (Kristianto, 2017).

In the case of inorganic coagulants, sweep coagulation typically occurs. This process takes place when metal coagulant doses are significantly higher than the solubility of the amorphous hydroxide which causes precipitation. The resultant precipitate then entraps the colloidal particles and can be removed from the suspension (Duan & Gregory, 2002). This method however is not preferable since large quantities of coagulants are required and excessive sludge production typically occurs which leads to high water treatment costs (Kristianto, 2017).

The fourth potential coagulation mechanism is polymer bridging which entails long-chain polymers being adsorbed onto particles (Yin, 2010). This then creates dangling coagulant polymer segments to bridge the particles. At high stirring speeds, strong flocs are produced by the bridging mechanism. However, in excessively turbulent conditions, polymer scission could lead to irreversible floc breakage. It is imperative that polymer doses be carefully controlled to ensure that there is sufficient uncopied colloidal particle surfaces available for polymer segment attachment. At doses exceeding the optimum, it is possible that colloidal particles can become re-stabilised because of steric repulsion amongst polymers on the particle surface. While at low doses, there will be insufficient polymer chains available to form bridges. Polymers that are linear and have a high molecular weight typically undergo polymer bridging. Organic plant-based coagulants typically undergo polymer bridging and charge neutralisation (Kristianto, 2017; Yin, 2010). The charge neutralisation and bridging mechanism under different dose conditions are depicted in Figure 7.

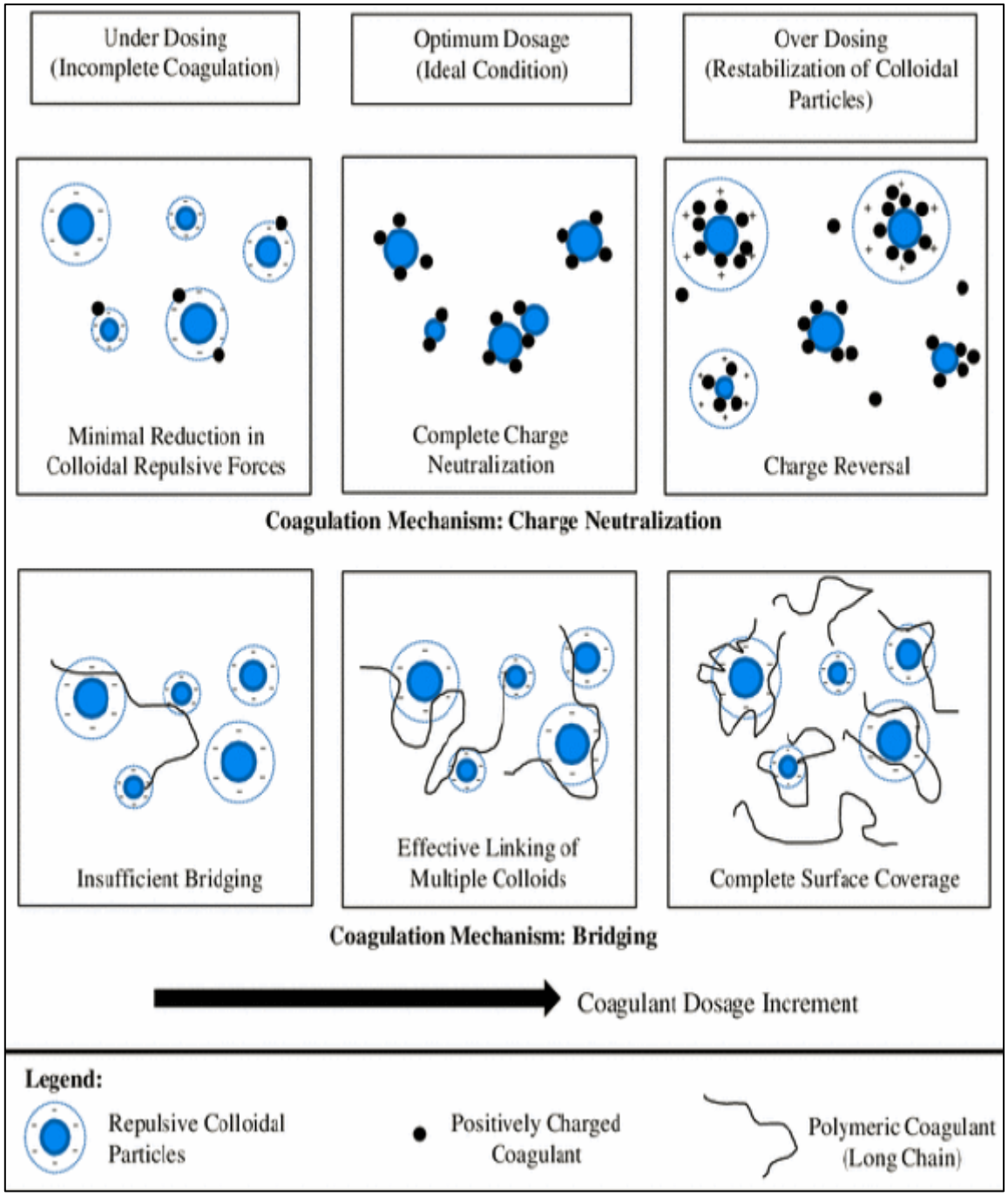


Figure 7: Charge neutralisation and bridging coagulation mechanism (Maheswari, et al., 2019)

The coagulation process is frequently applied for its efficient removal of natural organic matter and the prevention of the formation of disinfection by-products (Ncibi, et al., 2017). However, due to frequent changes in the concentration and composition of natural organic matter in water, conventional coagulation processes typically require exorbitant quantities of chemicals, which results in more sludge generation. Thus, this process needs to be optimised to improve efficiency. This can be accomplished by developing new coagulant agents and/or by adjusting the operating conditions.

2.3.1.1. Factors affecting coagulation

There are several operating conditions that affect coagulation efficiency and need to be carefully controlled in order to optimise the coagulation process.

2.3.1.1.1. Temperature

High temperature tends to increase the rate of reaction of the coagulant with the colloidal particles present in the raw water. Conversely, at a low temperature, the colloidal surfaces become stabilised to reduce the rate of the hydrolysis reactions and thereby potentially reduce the efficiency of coagulation (Tetteh & Rathilal, 2019).

2.3.1.1.2. pH

The pH needs to be well controlled during the coagulation process to ensure that neutralisation and agglomeration of flocs in solution is effective. An increase in pH causes the species to become charged which changes the reaction mechanism (Tetteh & Rathilal, 2019).

2.3.1.1.3. Mixing conditions

Mixing conditions influence the coagulation and flocculation process as it affects the average floc size distribution. Two mixing regimes, rapid mixing and slow mixing, are necessary to ensure successful coagulation and flocculation. Rapid mixing is carried out almost immediately after coagulant addition and is necessary to disperse the coagulant into the suspension and form a homogeneous solution. This mixing regime is especially vital in the treatment of highly turbid water since higher particle collision rates improve the floc formation process. It is important to optimise the rapid mixing time as well, because shorter fast mixing times result in bigger flocs and greater residual turbidity (BinAhmed, et al., 2015). Slow mixing is necessary to ensure that the particles remain in suspension for particle collisions to occur. Further, the slow mixing regime ensures a velocity gradient for particle collisions of particles that are of similar size and greater than 1 μm in size (Zhongguo, et al., 2013).

2.3.1.1.4. Coagulant dosage

Coagulant dosage is a key factor in ensuring effective coagulation and hence needs to be optimised. If the applied dosage is above the optimum level, then a potentially high water treatment cost may result due to excessive usage of a possibly expensive chemical. Further, overdosing may pose a health risk to humans, especially if the coagulant is toxic in high doses. On the contrary, a lower than optimum dose of coagulant would result in inefficient plant operation, since more treatment steps would be necessary to compensate for the ineffectiveness of the coagulant. Further, underdosing could potentially lead to the water being unsafe for consumption and thus being in violation of the South African National Standard for Drinking Water (Umgeni Water, 2016).

2.3.1.1.5. Coagulant aid

In certain cases, when even after an optimum coagulant dosage is applied, if the coagulation performance is still poor, a coagulant aid can be applied. Clay (typically bentonite, Fuller's earth, and kaolin) colloids provide nuclei to enable flocs to form and grow and add weight to the flocs. In some cases, clay is also beneficial for removing colour, odour and taste. Coagulant aids are typically employed as they can reduce the amount of coagulant needed, improve floc settling ease, enable flocculation of living organisms and because they have reduced pH sensitivity (Scholz, 2016).

2.3.1.2. Types of coagulants

Coagulants used in water treatment can be separated into two broad categories, namely inorganic and organic coagulants. Inorganic coagulants, particularly mineral salt coagulants, undergo the mechanism of charge neutralisation (formation of multi-charge complexes for adsorption of particulate matter) and sweep flocculation. The mechanism for organic coagulants however, involves charge neutralisation (adsorption of ions) and thereafter interparticle bridging, where one or more groups of the polymeric molecule adsorbs onto the particles in suspension and effectively destabilises the particles (Umgeni Water, 2016).

Inorganic coagulants such as aluminium sulphate have been conventionally used in water treatment, however the use of organic coagulants (particularly natural coagulants) has been recently researched and implemented due to factors such as its high turbidity removal efficiency, low-cost and biodegradable nature.

2.3.1.2.1. Inorganic coagulants

Inorganic coagulants can be classified into two categories: aluminium-based, and iron-based. Aluminium-based coagulants include aluminium sulphate (alum), sodium aluminate, polyaluminium chloride, and iron-based coagulants include ferric sulphate, ferric chloride, ferrous sulphate (copperas), and ferric chloride sulphate. The use of these coagulants is favoured for their enhanced adsorption characteristics, which is due to their ability to form multi-charged polynuclear complexes (IWA Publishing, 2020).

Upon the addition of inorganic coagulants, such as aluminium or iron salts, into a body of water, the coagulants dissociate into their ionic form (aluminium dissociates to Al^{+3}, and iron dissociates to Fe^{+3}), become hydrolysed and form positively charged complexes that can interact with negatively charged colloids (Ncibi, et al., 2017).

i. Aluminium-based coagulants

Aluminium-based coagulants have been conventionally utilised in water treatment for hundreds of years. Coagulants such as aluminium sulphate or alum ($Al_2(SO_4))_3$) and polyaluminium chloride (PACl) are favoured in water treatment systems for their enhanced ability to reduce levels of organic matter, microorganisms and turbidity (Srinivasan, et al., 1999). Aluminium sulphate is acidic in nature and requires the addition of a basic substance, such as calcium bicarbonate, to ensure efficient turbidity removal and minimal residual aluminium in the treated water. Polymeric coagulants such as polyaluminium chloride (PACl), aluminium chlorohydrate (ACH), and polyaluminium silicate sulphate (PASS) contain a higher basicity which reduces the need for an additional alkaline dose to adjust the pH. Further, these polymeric coagulants reduce the dissolved aluminium residual levels in the water and produces a more readily settleable floc than aluminium sulphate does (Brandt, et al., 2017). The process of water treatment using aluminium-based coagulants involves the addition of the aluminium salt, at an optimum pH and dose, to the water and thereafter carrying out flocculation, sedimentation and filtration (WHO, 2003).

Residual aluminium that remains in treated water poses major issues. High concentrations of aluminium may alter the cycling of nutrients such as phosphorous and dissolved organic carbon through absorption and may be toxic to aquatic life (Schecher & Driscoll, 1988). Aluminium in water also causes turbidity, reduces disinfection efficiency and may lead to the precipitation of $Al(OH)_3$ (Srinivasan, et al., 1999). Aluminium in treated water could also be linked to neuropathological diseases such as Alzheimer's disease and presenile dementia (Crapper, et al., 1973).

It is therefore important to incorporate a means of minimising residual aluminium content in water. According to WHO (2003), this can be achieved by ensuring adequate regulation of pH and dosage, sufficient mixing when adding the coagulant and improved filtration of the aluminium floc. Another issue faced by small-scale water treatment facilities, in maintaining a safe aluminium level of 0.1mg/litre or less, is that small-scale operation allows for less fluctuation in operation compared to larger facilities (WHO, 2003). Further, the lack of access to resources and expertise in smaller facilities prevents the determination of the most optimum and safe level of aluminium in the water. Besides industrial application, it then becomes infeasible for impoverished communities, who rely mainly on crude means of water treatment, to achieve this feat.

Although the benefits of using aluminium-based coagulants for water treatment are well recognised, the potential health and environmental concern associated with this chemical has given rise to the investigation into a safer, cheaper and more efficient means of water treatment.

ii. Iron-based coagulants

Iron-based coagulants include ferric sulphate, ferric chloride and ferrous sulphate. The ferric ions hydrolyse quickly to form different iron compounds that destabilise and aggregate colloidal particles for removal in later treatment steps. Iron coagulants perform effectively, over a wide range of pH and temperature conditions, in removing substances like trihalomethanes in soil in water. In general, iron-based coagulants have improved floc settling characteristics since it produces a denser floc than aluminium-based coagulants. Further, these iron-based coagulants pose a lower risk to human health than aluminium-based coagulants (Zouboulis, et al., 2007).

The use of ferric chloride is favoured in many industrial wastewater treatment facilities due to its high efficiency in the reduction of turbidity in water. Further, ferric chloride is preferred for its low cost as it is typically obtained as the waste material from steel-making operations. However, due to the presence of chloride ions in ferric chloride, it increases the corrosivity of the water (Brandt, et al., 2017). Due to the highly corrosive solution, handling equipment made of corrosive-resistant material, such as stainless steel, would need to be employed. Further, because of the potential of iron to stain and cause a brownish colour in water, specialised treatment would be necessary (Kerslake, et al., 1946). In order to combat the issue of corrosivity, polymeric ferric coagulants such as polyferric sulphate (PFS) are employed as an alternative. In addition to the lower corrosivity benefit, polyferric sulphate has been used to efficiently reduce turbidity over a wider range of pH than monomeric salts (Zouboulis, et al., 2007).

2.3.1.2.2. Natural organic coagulants

Due to the health concerns associated with inorganic coagulants, there is an increased need for a safer, and more environmentally friendly alternative. Thus, recent research has been focussed on the development of an effective bio-coagulant. The use of natural organic coagulants, such as chitosan and proteins extracted from plant matter, has served as a viable sustainable solution due to its non-toxicity, biodegradability and low-cost.

i. Non-plant-based coagulants

Polyelectrolytes, both synthetic and natural, have been employed as an effective means of coagulating colloidal particles in suspension and greatly improving the settling rates of flocs. Polyelectrolytes are long chain organics that can be anionic, cationic or non-ionic. In typical practice, organic anionic electrolytes are used for the treatment of sludge, and organic cationic electrolytes are used for coagulation purposes. The use of cationic polymers has been discouraged due to its potential toxicity to aquatic life, especially in water than has a low pH and alkalinity. Hence, the use of anionic polymers is preferred for its lower toxicity and fouling capability in water treatment plants.

Natural polyelectrolytes are becoming increasingly prevalent in water treatment due to its associated low toxicity to humans, since they are usually starch-based. Synthetic polyelectrolytes are valued as they can be applied in smaller doses (typically 0.05-0.25mg/l for polyacrylamide) than natural polyelectrolytes (typically 0.4 – 2.5mg/l for starch-based electrolytes), but still yield effective turbidity removal results. However, the dose of polyelectrolytes needs to be carefully controlled to avoid filtration issues such as 'mud ball' formation (Brandt, et al., 2017).

Natural coagulants such as polysaccharides have been investigated for their high shear stability, low-cost, ease of availability, low health risk to humans, environmentally friendliness, and wider dose range of applicability. Chitosan is an aminopolysaccharide that is derived from the deacetylation of chitin under alkaline conditions. Chitin is an abundant natural resource that is obtained mostly from fungi, endoskeletons of cephalopods and the cuticles of arthropods (Montembault, et al., 2005). Chitosan is favoured as a coagulant in water treatment for its biodegradability, non-toxicity and efficiency (Szyguła, et al., 2008). Alginate is a polysaccharide that is derived from marine brown algae and bacteria (such as *Azotobacter vinelandii* and *Pseudomonas aeruginosa*). Its use as a potential coagulating agent has been investigated due to its ability to form a strong gel structure with calcium ions (Devrimci, et al., 2012).

An important factor to consider when selecting a coagulant is cost and availability, especially if the treatment system is intended for rural communities. In a commercial sense, comparisons need to be made between the cost of the coagulant itself, processing stages and costs in different geographical regions. However, certain basic criteria can be utilised to aid selection of a suitable coagulant. An ideal coagulant would work efficiently over a wide range of pH, which would render pH correction redundant. Further, the coagulant would ideally need to be applied in low doses to save on material costs. Finally, the health and environmental impact of the coagulant needs to be a key factor in selection. Having fulfilled many of these criteria, the use of natural coagulants is an appealing means of water treatment (Saranya, et al., 2013).

ii. Plant-based coagulants

A plant-based coagulant refers to a natural, organic, ionic or non-ionic polymer that is derived from a plant constituent. These coagulants use the mechanism of charge neutralisation to aid the destabilisation of colloidal particles to form flocs, or polymer bridging to combine the colloidal particles using bridges. Plant-based coagulants have become increasingly researched and implemented for their biodegradability, availability, and non-toxicity benefits. The most thoroughly researched plant-based coagulant is produced using the protein extracted from the seeds of the *Moringa oleifera* plant (Ndabigengesere & Narasiah, 1998; Sasikala & Muthuraman, 2016; Choudhary & Neogi, 2017). Plants, like *Moringa oleifera*, have the potential to be cultivated and commissioned, not only commercially but in sectors that cannot afford, or do not have access to, specialised water treatment systems.

Even though there may be several potential plant candidates for water treatment, there are other factors that need to be considered when selecting a viable specimen. The following challenges typically exist when plant compounds are used to produce coagulants (Reinoso, et al., 2017).

- Lack of access or shortage in supply of raw materials
- Difference in growth conditions and seasonal variations in the harvesting of plants
- Potential toxicity (which makes it unsafe for consumption)
- Low availability and abundance of the plant material
- Environmental pollutants contaminating plant extracts
- Challenges in the extraction and purification of plant extracts due to complex plant chemistry

Moringa oleifera

Background

Moringa oleifera is a tropical plant belonging to the family of *Moringaceae*. *Moringaceae* is commonly referred to as the "drumstick" or "horseradish" family. The *Moringa oleifera* plant has also been dubbed the "miracle tree" due to its phenomenal healing abilities for several illnesses. Thus far, 13 species have been identified in tropical and subtropical regions such as Southwest Asia, Southwest Africa, Northeast Africa and Madagascar (Rani, et al., 2018). *Moringa oleifera* can be grown in a wide range of climates, typically in temperatures ranging from 25 ˚C to 40 ˚C. However, the plant can survive in temperatures as low as 5 ˚C and as high as 50 ˚C (Al Taweel & Al-Anbari, 2019).

The *Moringa oleifera* plant is an angiosperm that native to India where it is cultivated and well-studied for its medicinal, nutritional and various other benefits. *Moringa oleifera* is a fast-growing, slender evergreen tree with drooping branches. The plant grows up to approximately 10 meters in height. Flowers and fruits, in certain climates can be produced throughout the year (Bichi, 2013). *Moringa* seeds are typically round in shape and have a semi-permeable seed hull with three papery wings. Each seed weighs approximately 0.3g (Adegbe, et al., 2016). Several parts of the *Moringa oleifera* plant, like the leaves, roots, seed, fruit and bark contain substances have beneficial properties, which makes the plant useful in pharmaceutical, agricultural and water treatment industries (Bodlund, 2013). Figures 8 and 9 show *Moringa oleifera* plant components.

Figure 8: *Moringa oleifera* plant (Ali & Kemat, 2017)

Figure 9: *Moringa oleifera* seeds (Ali & Kemat, 2017)

Uses and advantages

Moringa oleifera plant constituents have many nutritional benefits. Thus, many studies have been carried out to investigate the potential applications of its bioactive components. The leaves of this plant are rich in vitamins and minerals like calcium, potassium, magnesium, zinc, and Vitamins A, B, C, D and E, which make the plant an excellent nutritional supplement (Mbikay, 2012). The leaves also have phytochemicals (such as flavonoids, tannins and sterols) and are low in calories. Phytochemicals in plant-based foods have been found to improve the overall health of people with diabetes and were found to reduce high blood pressure and cholesterol levels. Flavonoids present in the leaves have excellent antioxidant and anti-inflammatory properties and anti-carcinogenic and anti-mutagenic activities. Further, tannins are capable of inactivating and destroying microorganisms (Kasolo, et al., 2010). The Moringa plant has been utilised to treat ailments such as skin infections, blackheads, blood impurities, anaemia, anxiety, asthma, bronchitis, and cholera. This plant also has value in the cosmetic industry where it is found as a constituent in hair and body moisturisers and conditioners (Razis, et al., 2014). Moringa seed oils is also commonly used for topical skin application (Kleiman, et al., 2008).

The dried and ground seeds and roots of the *Moringa oleifera* plant have been used in some cultures as a base for seasoning sauces. Since the seed kernels contain approximately 42% oil, it is processed and used in many industries. The seed oil does not easily deteriorate; therefore, it is often used as a lubricant for machinery (such as time pieces). The high oleic acid content of the seed oil makes it suitable as vegetable cooking oil. The seed oil is also useful as a stabilizing scent in the perfumery industry since it has the ability to absorb and retain volatile components. The Moringa seed husk has been investigated as a possible feedstock for biodiesel production due to its biodegradability and minimal environmental pollution (Rashid, et al., 2011). Active components present in leaves can be extracted and used as a growth hormone for young plants; it has also been found that the use of this growth hormone on young plants has increased pest and disease resistance, and improved the fruit yield of plants (Adegbe, et al., 2016).

There has been an increasing need for safe and clean drinking water, especially in developing nations with limited access to high-end water treatment systems. Due to the high costs associated with water treatment equipment and chemicals (such as aluminium sulphate and polyelectrolytes), locally sourced biomaterials have been investigated as water treatment coagulating agents. The *Moringa oleifera* seed protein, which was salt extracted (using 1M NaCl), was able to remove 99.8% of the turbidity from water with an initial turbidity of 450 NTU. Further, up to 100% removal of iron and manganese and up to 96% of *Escherichia coli* bacteria was removed from turbid water(with turbidities between 50- 450 NTU) by treatment with *Moringa oleifera* (Nkurunziza, et al., 2009). *Moringa oleifera* seeds have therefore been traditionally used for water treatment in households in locations such as rural Sudan and West Asia due to its excellent turbidity removal, disinfection and softening ability (Bichi, 2013). According to Okuda & Ali (2019), *Moringa oleifera* can be applied as a coagulant with little pre-treatment that does not require harmful chemicals. Further, minimal adverse impact to human health occurs if this natural coagulant is applied in a slight overdose. An additional benefit of using *Moringa oleifera* is that the produced coagulant sludge can be used as a feedstock for animals and as a fertiliser for plants (Okuda & Ali, 2019). This plant is therefore one of the most widely studied bio-coagulant due to its ability to remove turbidity in water with high efficiency and in a low-cost and environmentally friendly manner.

Protein structure

Shelled *Moringa oleifera* seeds contains about 37% proteins, 35% lipids, and 5% carbohydrates. Un-shelled seeds consist of approximately 27% proteins, 21% lipids, and 5.5% carbohydrates (Ndabigengesere, et al., 1995). The active coagulating agents of *Moringa oleifera* seeds were found by Ndabigengesere & Narasiah (1998) to be dimeric cationic proteins that have an approximate molecular weight of 13kDa and an isoelectric point between 10 and 11. There are two main protein components that have been identified from the *Moringa oleifera* seed extract: $MO_{2,1}$ and *Mo*-CBP_3(major isoforms are *Mo*-CBP_3-3 and *Mo*-CBP_3-4). Both proteins have good thermostability and retain coagulation and antifungal properties after heat treatment (Moulin, et al., 2019).Through, amino acid analysis of $MO_{2,1}$(which has a molecular mass of 6.5kDa), it was established that the major amino acids constituents are glutamine, proline and arginine (Gassenschmidt, et al., 1995). *Mo*-CBP_3 has a molecular mass of 14kDa and is thermostable and antifungal (Freire, et al., 2015). Upon amino acid analysis, *Mo*-CBP_3 was found to be a part of the 2S albumin family, which is characterised by a compact fold of amino acid chains. This type of arrangement gives proteins marked stability and heat resistance (Moulin, et al., 2019).

The Moringa seed proteins studied by Broin, et al. (2002), which had a molecular weight of 6kDa, were found to contain eight positively charged amino acids (7 arginines and 1 histidine) and one negatively charged amino acid (aspartic acid). Other amino acids that were identified in Moringa seeds are glutamic acid, methionine, and proline (Jahn, 1988). Since the proteins contain mostly positively charged amino acids, the proteins in solution are highly positively charged (also indicated by its pI of 12.6) (Broin, et al., 2002). The major proteins found in *Moringa oleifera* seeds are globulin and albumin, which have protein fractions of 53% and 43% respectively. Other proteins contained in Moringa seeds include prolamin and glutelin (Baptista, et al., 2017).

Mechanisms of action

The coagulation mechanism of Moringa proteins is either adsorption and neutralisation of charges, or adsorption and bridging of destabilised particles; it is also possible that both of these mechanisms could also be taking place simultaneously. However, the predominant mechanism for coagulation using *Moringa oleifera* proteins was identified as adsorption and charge neutralisation (Ndabigengesere, et al., 1995).

The $MO_{2,1}$ *Moringa oleifera* seed protein has been proposed to undergo coagulation via a patch charge mechanism. This mechanism involves the positively charged proteins becoming bound to the surface of negatively charged particles by electrostatic interactions. Consequently, negatively and positively charged parts form on the particle surfaces. Further, some regions on the particle surface become neutralised. The reduction in electrostatic repulsion between the negatively particles then causes the particles to become more prone to coagulation, which subsequently leads to the formation of flocs (Gassenschmidt, et al., 1995).

Flocculation can be described by the ‘bridge formation model’. Flocculation occurs due to binding of positively charged macromolecules to the surface of negatively charged particles by Coulomb forces and due to neutralisation of part of the surface charge. Agglomeration takes place because of the reduced electrostatic repulsion. Since only a small portion of the positively charged macromolecule attaches to the surface of one particle, the rest of the macromolecule can bind to the surface of another particle. Consequently, agglomeration and floc formation result due to the bridges that form between the negatively charged particles (Gassenschmidt, et al., 1995). Figure 10 provides a visual depiction of the coagulation, flocculation and sedimentation process that occurs when *Moringa oleifera* is applied as a coagulant.

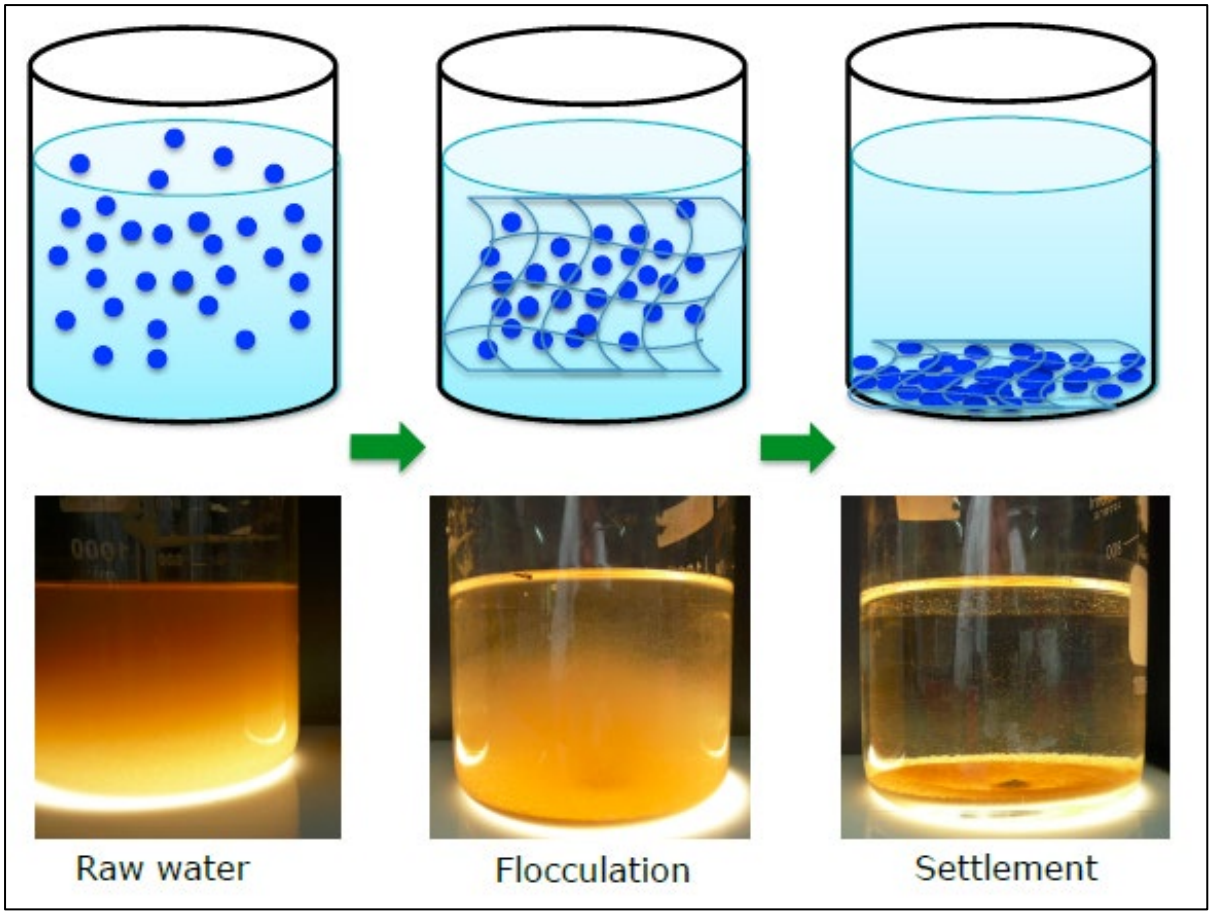


Figure 10: Coagulation, flocculation and settlement mechanism of *Moringa oleifera* protein (Bodlund, 2013)

Performance in water treatment

It was found that salt-extracted (using 1M NaCl) *Moringa oleifera* coagulants could achieve a turbidity removal efficiency that was seven times higher than that achieved by normal aqueous extraction (using distilled water). Further, salt-extracted Moringa coagulants could reduce the turbidity of a 50 NTU turbid kaolin solution by more than 95%, using a coagulant dose of 4ml/l; whereas the aqueously extracted coagulant could reduce the turbidity of a 50 NTU turbid kaolin solution by 78%, at a much higher dose of 32ml/l (Okuda, et al., 1999). Maximum protein extraction, using NaCl salt solutions as the extraction medium, of 84.72% from defatted *Moringa oleifera* seed flour was achieved in a study carried out by Singh, et al. (2011). Baptista, et al. (2017) determined that the protein extractability of the Moringa seed is 98% of the total protein content, when extracted using aqueous, saline (using NaCl), alcoholic (using ethanol) and sodium hydroxide extraction.

Although *Moringa oleifera* generally showcases excellent performance as a coagulant, there are limitations to its use. In a study by Awad, et al. (2013), a high homogeneous coagulant dose of 600ml/l (of a 10g/l coagulant stock solution) was required to achieve 90% turbidity removal from an initial turbidity of 32 NTU. It was found that the high coagulant dose led to the Moringa particles increasing the organic load of the treated water which could lead to negative impacts such as colour, odour, taste and microorganism presence. In addition, this study found that at a settling time of 30 minutes (after coagulation-flocculation processes), the turbid water had high quantities of suspended flocs which implies that flocs formed at this time were small or not dense enough to settle. Awad, et al. (2013) also found that after five hours of settling, 64.2%, 95.9%, and 98.4% turbidity removal could be achieved in water samples with low (29.6 NTU), medium (236 NTU) and high (543 NTU) initial turbidities. This implies that high settling times are required for effective turbidity removal and that this plant species is most effective in highly turbid waters.

In a study by Dezfooli, et al. (2016), *Moringa oleifera* seed proteins were purified by firstly removing seed oil, carrying out salting-out (using ammonium sulphate), performing dialysis and finally using heat treatment. The combination of different techniques for purification in this study was found to be relatively simple. The heat treatment served to not only purify proteins but also to deactivate microorganisms and enzymes, to allow for a longer preservation time of the coagulant proteins.

Neutron reflection was utilised in a study by Kwaambwa, et al. (2010), to investigate the adsorption of Moringa proteins onto silica oxide, where strong binding was noted even at low protein concentrations. In a comparative study of Moringa protein adsorption onto alumina and silica surfaces, it was found that proteins adsorbed onto alumina were not strongly bound onto the surface, whereas Moringa proteins were irreversibly bound to silica surfaces (Kwaambwa, et al., 2015). *Moringa oleifera* seed protein extracts have been adsorbed onto silica surfaces (in combination called f-sand) and used to treat water of varying hardness (Nordmark, et al., 2018). In this study, ellipsometry was utilised to establish adsorption isotherms for this particular process. Nordmark, et al. (2018) concluded that the protein-modified sand would be stable upon reuse for water filtration making it an effective and sustainable water purification device. *Moringa oleifera* cationic proteins (MOCP) studied by Jerri, et al. (2012), were immobilised onto sand granules and thereafter rinsed to remove excess organic matter to prevent bacterial growth in the treated water. The f-sand was able to reduce turbidity (in a kaolin clay solution), remove silica microspheres and pathogens from water, and render *Escherichia coli* bacteria non-viable. Since the immobilised proteins had the ability to retain its turbidity removal and anti-bacterial properties after functionalisation treatment, it was concluded that the process of Moringa protein adsorption onto sand will prove to be a sustainable means of producing storable drinking water (Jerri, et al., 2012). Adsorption of *Moringa oleifera* seed proteins onto nanosilica has also been investigated to produce an efficient and eco-friendly material(called ProMNS) to remove antibiotic pollutants(particularly ciprofloxacin(CFX)) from hospital wastewater (Pham, et al., 2020).

Glycine max

Background

Glycine max, commonly known as soybean or soya bean, is an annual plant that belongs to the *Fabaceae* family. This species is native to East Asia and is also widely distributed in Australia and Africa (Badole, et al., 2015). *Glycine max* is produced throughout South Africa, with the greatest producing regions being Free State and Mpumalanga (Sihlobo & Kapuya, 2016). The plant optimally grows in regions with mean annual temperatures of 20-30 ˚C. The versatile plant can grow in a wide range of soils but prefers moist, fertile and well-drained soils for optimum growth (Lim, 2012). Better yield and seed quality are usually achieved when rainfall is in the range of 500-900mm. *Glycine max* is one of the most economically important plant species and serves as a source of high-quality protein and vegetable oil for animal and human nutrition (Department of Agriculture, Forestry and Fisheries, 2010).

Glycine max is a leguminous plant that is bushy and erect (shown in Figure 11). This plant is extensively branched, has well-developed roots and grows to a height of 40-100cm. The leaves of the *Glycine max* plant are alternate, have hair in some cases and vary in shape. *Glycine max* produces many small pods that encapsulate one to four, round to oval, yellow to black seeds (Badole, et al., 2015). Pale yellow seeds are generally deemed commercially acceptable for human consumption and oil production.

The crop estimates committee (CEC) has estimated that 730 500 hectares of soybeans have been planted in South Africa in the 2018/19 marketing year (USDA Foreign Agricultural Service, 2019). *Glycine max* production efforts in South Africa are aimed at increasing the crop output, predominantly for the manufacture of animal feed for the livestock sector (especially pork, beef and poultry). Oil produced from *Glycine max* and direct human consumption of the legume(as soups and soymilk products) forms a small part of the South African household diet (Dlamini, et al., 2014).

Figure 11: *Glycine max* plant (Lim, 2012)

Figure 12: *Glycine max* seeds (Lim, 2012)

Uses and advantages

Glycine max is one of two known plant foods that contain all essential amino acids that are similar to those amino acids found in meat. Further, the seed (shown in Figure 12) has a high fibre and protein content, is low in saturated fat, and is cholesterol- and lactose-free. *Glycine max* is also an excellent source of omega-3 fatty acids, vitamins (such as vitamin E) and minerals (such as calcium and iron).

Unripe seeds of *Glycine max* are typically eaten as a vegetable and the dried seeds tend to be eaten whole or sprouted. Further, the seeds can be processed into soy milk, which has several uses such as for the production of curds, cheese and a protein supplement for infants. Soy sauce is also commonly produced from mature fermented seeds and is a popular condiment. In addition, soy bean flour can be defatted and utilised in bakeries and as additives and extenders for meat products and cereal flour (Department of Agriculture, Forestry and Fisheries, 2010).

The pharmacological properties of *Glycine max* are vast, mainly due to the presence of isoflavones in the plant. Due to the high polyunsaturated fat, fibre, vitamin and mineral content and low saturated fat content, *Glycine max* has been reported to be beneficial in the prevention of cardiovascular diseases. Further, the isoflavones in the plant can be used in the treatment of estrogen-deficient diseases (like menopausal symptoms). Populations that have a diet that constitutes mostly of *Glycine max* protein rather than animal protein, were found to have a lowered risk of contracting prostate and breast cancers compared to other populations. Evidence has also been gathered on the benefits of using dietary phytoestrogens (like soy isoflavones) for osteoporosis prevention, as an alternative to hormone-replacement therapy. Further, a combination of soy isoflavone with moderate exercise has been proven to prevent body fat accumulation, making it ideal for the prevention of obesity and its associated diseases. *Glycine max* boasts a host of several other beneficial properties and can be used as an antioxidant, antidiabetic, anti-hypercholesterolemic, antimicrobial, and anti-skin aging agent. Traditional applications of *Glycine max* include its use in the remedy of the heart, liver, kidney, and stomach ailments. It has also been utilised in the treatment of the common cold, diarrhoea, beriberi, habitual constipation, leg ulcers, iron deficiency anaemia, and skin disease (Lim, 2012).

Industrial use of oil derived from *Glycine max* includes the manufacture of paints, linoleum, printing inks, oilcloth, disinfectants and insecticides. The by-product of soy oil, lecithin phospholipids, is applied as a wetting and stabilizing agent in the cosmetic, food, pharmaceutical, and detergent industries. Soy meal is used as livestock feed, especially in the poultry industry, due to its high protein content (Department of Agriculture, Forestry and Fisheries, 2010). Soy meal and soybean has also been applied in the manufacture of synthetic adhesives and fibre. Biodiesel, for use as fuel in vehicles or generators, can also be produced from *Glycine max* and is a renewable and environmentally friendly alternative to petroleum diesel (Lim, 2012).

Protein structure

Dried *Glycine max* seeds contain approximately 40% proteins, 18-22% lipids and 35% carbohydrates, along with other bioactive compounds (Badole, et al., 2015). Soybean flours contain 45.4% protein, 41.5% carbohydrates and 0.6% lipids. While salt extracted isolates contain 72.6% protein, 13.7% carbohydrates and 10.1% lipids (Karaca, et al., 2011).

The seed proteins of *Glycine max,* like all legumes, are albumins and globulins. The major storage protein(globulins) in *Glycine max* are glycinin(11S) and β-congylycinin(7S). Salt addition tends to favour protein aggregation and precipitation in mixtures that contain a higher proportion of 11S proteins. (Tay, et al., 2006). Through protein fractionation, it was established that *Glycine max* seed proteins comprise 30.2- 46.5% globulin, 15.7-31.8% albumin, 16.1-38.5% glutelin and 4% prolamin (Ciabotti, et al., 2016).

Glycine max proteins have different isoelectric points, molecular weights, gel-forming properties and thermal transition temperatures. β-congylycinin is a trimeric protein that has a molecular weight of 126-171kDa (Deak, et al., 2006) and an isoelectric point of 4.9 (Salas, et al., 2012). Glycinin is a quaternary structure that has a molecular weight of approximately 320-350kDa and an isoelectric point of 4.64 (Salas, et al., 2012). Overall, *Glycine max* was reported to have an isoelectric point in the range of 4 to 5, making it negatively charged at a neutral pH (Jaramillo, et al., 2011). The contributions of these proteins to emulsification and foaming properties have played a role in food formulations.

Performance in water treatment

The effectiveness of raw *Glycine max* as a natural coagulant was investigated and compared to aluminium sulphate in a study by Mbogo (2008). Aqueous extraction was utilised to recover the active components from the seeds of *Glycine max.* The coagulant was able to reduce an initial turbidity of 482 NTU to 5.63 NTU (98.8% turbidity removal). This was achieved at an optimal dosage of 1000mg/l. However, when used as a coagulant aid (80% natural coagulant and 20% aluminium sulphate), *Glycine max* was able to achieve 100% turbidity removal (from the same initial turbidity) at a coagulant dose of 600mg/l.

In a study by Hussain & Haydar (2020), raw and defatted *Glycine max* seeds were tested as coagulants and compared to aluminium sulphate. The results showed that aqueously extracted active components from *Glycine max* could reduce an initial turbidity of 200 NTU by 60-97.4% while aluminium sulphate reduces turbidity by 75.1-98.9%. Defatted *Glycine max* produced turbidity removal results of 72-98.4%, which was comparable to aluminium sulphate. This study investigated the optimum conditions for each specimen and noted that in a pH range of 3-6 and dose range of 20-60mg/l, >95% of turbidity can be removed using raw *Glycine max.* In a similar study, *Glycine max* seed extracts were able to reduce an initial turbidity of 135 NTU by 97.6% at an optimum dose of 25mg/l (Maheswari, et al., 2019).

Glycine max protein showcases an increase in solubility with increasing ionic strength. This was evidenced by the findings of Yuan, et al. (2002), which depicted the lowest solubility for both 7S and 11S proteins at 0M NaCl (aqueous extraction), and the highest solubility at 0.3M NaCl(salt-extraction). Deak, et al. (2006) reported that at an optimum salting-in concentration of 250mM, 75.1% of *Glycine max* proteins can be extracted, with β-congylycinin constituting 70% of the storage proteins extracted. This study also reports that at a salt concentration of 500mM NaCl, maximum yields of β-congylycinin protein and solids were obtained.

The adsorption of *Glycine max* proteins onto solid substrates is complex and is dependent on the physicochemical environment. Glycinin proteins were established to have a high affinity for substrates like cellulose and silica. *Glycine max* protein adsorption onto silica was found to increase with increasing ionic strength (Salas, et al., 2012). The proteins undergo association-dissociation in solution based on the presence of electrolytes. At low ionic strengths (less than 0.1M NaCl), the protein dissociates and at high ionic strengths (greater than 0.5M NaCl), the dissociation reduces. Figure 13 depicts a possible surface arrangement of *Glycine max* protein molecules onto a substrate. At low ionic strengths it is anticipated that a more extended layer of proteins occurs, while at high ionic strengths, screening of electrostatic interactions results in a more compact adsorbed protein layer (Salas, et al., 2012).

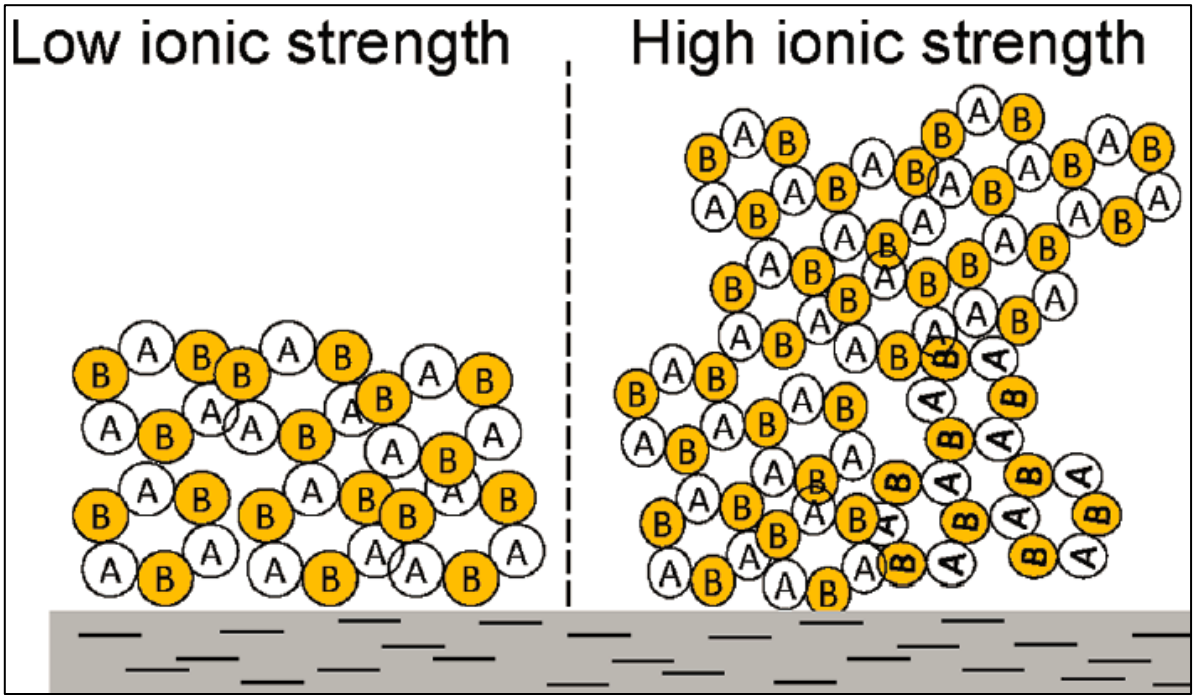


Figure 13: Illustration of how *Glycine max* protein adsorbs onto a negatively charged substrate (Salas, et al., 2012)

Cicer arietinum

Background

Cicer arietinum, commonly referred to as chickpea or Bengal gram belongs to the Fabaceae or Leguminosae family. The plant is cultivated extensively in India, Pakistan, Turkey, Mexico and Ethiopia (Lim, 2012). *Cicer arietinum* is relatively drought-resistant but grows preferably on a sunny site in a cool, dry climate. The crop grows in a wide range of soils, from sandy to sandy loams and black cotton soils. Annual rainfall of 600-1000mm is preferred by this plant and it grows optimally at day temperatures of 18-26 ˚C and night temperatures of 21-29 ˚C.

Cicer arietinum is an erect, branched annual herb that grows up to 1 meter tall (as shown in Figure 14). The plant has deep roots, glandular stems, and alternate leaves. The fruit is an inflated rhomboid-ellipsoid pod (approximately 14-30mm by 8-20mm) and contains 1 to 4 seeds. These seeds(depicted in Figure 15) have a prominent beak, have a smooth or wrinkled surface, and are creamy to dark brown in colour (Lim, 2012). The size, shape and colour of the seed however varies with cultivars (Paredes-Lopez, et al., 1991).

Cicer arietinum is an important crop and has a production ranking of third in the world, with a production rate of 11.67 million tons annually (Merga, et al., 2019). It is the second most widely grown crop in the world (after *Glycine max*) and plays an important role in food security (Zin, et al., 2018). The crop has the potential to become increasingly used for its desirable functionalities (high protein content and digestibility) and relatively low cost (Chang, et al., 2012).

Figure 14: *Cicer arietinum* foliage and pods (Lim, 2012)

Figure 15: *Cicer arietinum* seeds (Lim, 2012)

Uses and advantages

Cicer arietinum is typically consumed as a dry pulse. The seeds are utilised in stews, curries, soups, porridges and salads. Further, the seeds can be ground into a flour which is used as a coating for meat and vegetables prior to frying. The roasted seed has also been employed as a coffee substitute. The seed sprouts, young shoots, young seed pods and leaves can be consumed as a vegetable. *Cicer arietinum* seeds serve as a good source of protein, carbohydrates, dietary fibre, folate, niacin, vitamins A and β-carotene and minerals like calcium, iron, potassium, magnesium, zinc, phosphorous and copper. Amino acids from protein isolates of *Cicer arietinum* were able to achieve the FAO/WHO essential amino acid requirements for pre-school children. Further, in-vitro digestibility of *Cicer arietinum* was found to exceed that of *Glycine max* (Lim, 2012).

Biochanin A and formononetin, which are isoflavones found in *Cicer arietinum,* were established as estrogenic and showcased hypolipidemic activity. Further, this plant was deemed effective in reducing cholesterol levels. In terms of anti-obesity and antidiabetic activity, *Cicer arietinum* was found to reverse visceral adiposity, insulin resistance and dyslipidaemia. Evidence also shows that *Cicer arietinum*-derived peptides have the ability to inhibit the growth of colon tumours. In traditional medicine, *Cicer arietinum* has been used in the treatment of diarrhoea, bronchitis, cholera, snakebite, sunstroke, and constipation. It has also been found to be effective in the treatment of skin disease (Lim, 2012).

Industrial applications of *Cicer arietinum* include the production of protein shampoo for normal/dry hair, baby shampoo and revitalizing hair oil. *Cicer arietinum* seeds and seed husks are also used as horse and poultry feed (Lim, 2012). In a study by Zin, et al. (2018), *Cicer arietinum* was utilised as a coagulant aid (4g/l aluminium sulphate and 0.6g/l natural coagulant) in the treatment of leachate and was able to remove 94% of colour at an optimum pH of 6. Individually, *Cicer arietinum* and aluminium sulphate were able to reduce colour by 22% and 88% respectively.

Protein structure

Raw *Cicer arietinum* flours contain 16.7% protein, 71.1% carbohydrates, and 3.8% lipids. Protein isolates produced by salt extraction contain 81.6% protein, 7.2% carbohydrates and 0.6% lipids (Karaca, et al., 2011). Albumin, prolamin, glutelin and globulin content ranged from 8.4-12.3%, 19.4-24.4%, 3.1-6.9% and 53.4-60.3% respectively. By amino acid analysis, the most prevalent amino acid in *Cicer arietinum* was glutamic acid, followed by aspartic acid and arginine (Lim, 2012).

Cicer arietinum seed proteins (which compose 25.3-28.9% of the total seed content) consist of 56% globulins, 12% albumins, 18.1% glutelins, 2.8% prolamins and residual protein. The relatively high glutelin content for a legume makes *Cicer arietinum* unique. The globulin constitutes 11S legumin and 7S vicilin, which have molecular weights of 320-400kDa and 145-190kDa respectively. *Cicer arietinum* 2S albumin is approximately 20kDa in molecular weight and is made up of two polypeptides with molecular weights of 10kDa and 12kDa (Chang, et al., 2012). *Cicer arietinum* has an isoelectric point of 3.7 making it negatively charged at a neutral pH (Jaramillo, et al., 2011).

Performance in water treatment

In a study carried out by Asrafuzzaman, et al. (2011*), Cicer arietinum* proteins were aqueously extracted and applied in a jar test to establish its turbidity removal efficiency. The results from this study showed that, at an optimum dose of 100mg/l, at three initial turbidity levels, high (95 NTU), medium (49 NTU) and low (31 NTU), *Cicer arietinum* was able to reduce the initial turbidity by 95.9%, 81.6% and 71.3% respectively. The turbidity removal of 95.9% from a high initial turbidity shows that *Cicer arietinum* is almost as effective as aluminium sulphate as a coagulant. Further, in this study, *Cicer arietinum* was proven to be a more effective coagulant than *Moringa oleifera* and *Dolichos lablab* at the turbidity levels and doses tested.

Cicer arietinum seeds were tested as a natural coagulant for the treatment of turbid water with an initial turbidity of 390 NTU (Priya & Sharpudhin, 2016). In this study, a range of doses (0.05-0.25g/ml) and pH (5-9) were tested. At an optimal dose of 0.15g/ml and pH of 7, turbidity was reduced by 84.3% (from 390 NTU to 61.2 NTU). *Cicer arietinum* was also able to reduce the total dissolved solids by 30.3% and chromium content by 66.7%. In a similar study by Maheswari, et al., (2019), dried *Cicer arietinum* seeds were able to achieve 97.3% turbidity removal (from an initial turbidity of 135 NTU) at an optimum dosage of 25mg/l.

In another study, *Cicer arietinum* was tested as a natural coagulant for the treatment of three turbidity levels (50 NTU, 150 NTU and 250 NTU). Aqueously extracted active components were added to the turbid samples in doses ranging from 10-40mg/l. At 250 NTU, *Cicer arietinum* was able to reduce the turbidity by 82% at an optimum dose of 30mg/l. The coagulant however did not perform well at initial turbidities of 150 NTU (where a maximum of 31.3% turbidity removal was achieved) and 50 NTU (where an increase in turbidity was observed). In a similar experiment using *Cicer arietinum* as a natural coagulant, 74% turbidity removal from an initial turbidity of 80 NTU, 76% turbidity removal from an initial turbidity of 140 NTU and 77% turbidity removal from an initial turbidity of 210 NTU was achieved at doses of 26mg/l, 57.2mg/l and 43mg/l respectively (Hossain, et al., 2018).

Cicer arietinum and aluminium sulphate were tested as coagulants for the removal of turbidity and colour. Various doses(70-100mg/l), at a fixed pH level of 7, were investigated for this purpose. At a dose of 90mg/l, *Cicer arietinum* was able to achieve 98% turbidity removal (from an initial turbidity of 200 NTU) and 94.7% colour removal. Therefore, *Cicer arietinum* performed better than aluminium sulphate, which could achieve 97.5% turbidity removal and 93.9% colour removal at the same dose (Jaramillo & Epalza, 2019). In a similar study, a coagulant, composed of 80% *Cicer arietinum* and 20% aluminium sulphate, was able to reduce the turbidity of 250 NTU by 96.8% at a dose of 300mg/l (Gurumath & Suresh, 2019).

The effects of ionic strength on *Cicer arietinum* protein solubility was investigated by Osman, et al. (2005). The study revealed that protein solubility at a sodium chloride concentration of 0.2M was less than that achieved at 0M. Findings also show that the protein solubility increases with increasing ionic strength up to a maximum of 80%, at an optimum NaCl concentration of 0.4M, due to the salting-in effect. It thereafter decreases with increasing salt concentration due to the salting-out effect. An increase in salt concentration also increased the emulsion capacity, foaming capacity, and foam stability of the proteins.

Hui (1996) found that more proteins can be extracted by salt extraction using either NaCl or K_2SO_4 (61.9-77.2%) than by aqueous extraction using water (43-49%). Salt extraction produced greater quantities of globulin proteins while aqueous extraction produced majorly albumin fractions. However, 5.2-21% of the proteins recovered by aqueous extraction were globulins, possibly resulting from the high salt content of the protein itself which could have increased the ionic strength in solution which thereby favoured globulin extraction.

Vigna unguiculata

Background

Vigna unguiculata (also known as cowpea or black-eyed pea), belongs to the Fabaceae family. Figures 16 and 17 show plant components of *Vigna unguiculata. Vigna unguiculata* originated in Africa but is currently cultivated across Asia and the Pacific, Southern Europe, Southern United States, and the Middle East (Lim, 2012).It is an annual legume plant that prefers warm and dry conditions (at temperatures greater than 0˚C, preferably between 25 ˚C -35 ˚C). The crop also grows optimally in well-drained, sandy looms and soils (Jayalakshmi, et al., 2017). However, *Vigna unguiculata* can tolerate strongly alkaline or acidic soils better than other legumes (Lim, 2012). The flowers of *Vigna unguiculata* are majorly self-pollinating and seeds are carried in pods. The plant is also drought-resistant making it suitable for use in developing nations that experience low to no rainfall (Jayalakshmi, et al., 2017).

Vigna unguiculata is a leguminous and herbaceous annual crop. The leaves of this plant are alternate (5cm to 16cm long) and stems grow up to 3m long and are slightly ribbed. The flowers are white, greenish yellow or purple. The crop contains long, cylindrical pods containing seeds that are approximately 6-12mm long. The seeds themselves can be smooth or wrinkled and coloured cream (Lim, 2012).

Vigna unguiculata is ranked the third most produced legume worldwide, and in 2004 was grown on 10.7 million ha. In Turkey, the crop was cultivated on 2925 ha and had a total production and average yield of 2937t and 1004kg/ha respectively in 2006 (Düzdemir, et al., 2009).

Figure 16: *Vigna unguiculata* plant (Lim, 2012)

Figure 17: *Vigna unguiculata* seeds (Lim, 2012)

Uses and advantages

Vigna unguiculata is a food staple in several African countries, making up greater than half of the plant protein in human diets. The roots, flowers, leaves, pods and seeds are regularly consumed in these countries (Lim, 2012). The crop is an excellent source of calories, minerals and vitamins and serves as a valuable source of dietary protein (containing 18-35% protein). Especially in areas with chronic protein shortage, *Vigna unguiculata* provides ample nutritional value to humans and domestic animals. Since the seed is a great source of protein, it is utilised as a flour for products like baby formula or as a supplement for children in pre-school. Other *Vigna unguiculata*-derived products include pasta, baking products or extruded products (Khalid, et al., 2012).

Vigna unguiculata protein isolates showcased good functional properties like emulsifying, foaming and solubility activities (Lim, 2012). In terms of health benefits, extracts from *Vigna unguiculata* seeds were used in the treatment of breast cancer. Further, several proteins found in *Vigna unguiculata* seeds were found to have antiviral and antifungal properties and were capable of inhibiting human immunodeficiency virus. *Vigna unguiculata* leaf extracts have inhibitory effects on fungal and bacterial pathogens. In traditional medicine, leaves and seeds have been used to treat skin swelling and infections. Leaves were utilised to treat burns, headaches, and tooth disorders (Lim, 2012).

Vigna unguiculata roots were also traditionally utilised as an antidote for snakebites and for the treatment of constipation. The seed powder was also applied as a relief of insect stings and to destroy worms in the stomach (Lim, 2012). In Tanzania, *Vigna unguiculata* is utilised as a food crop and for animal fodder (Jayalakshmi, et al., 2017). *Vigna unguiculata* is also used to enrich soil with nitrogen, since it has nitrogen-fixing nodules that harbour bacterium (Lim, 2012).

Protein structure

The active components of *Vigna unguiculata* consist of at least two different coagulant proteins that have a molecular weight of approximately 6kDa (Jayalakshmi, et al., 2017). Proximate composition of *Vigna unguiculata* reveals that the seed contains 2.5-31% protein, 56-65.7% carbohydrates and 1.1-3% fat (Lim, 2012). Plant seed proteins constitute 66.6% globulin, 24.9% albumin, 4.7% glutelin and 0.7% prolamin (Agustin, et al., 2020). The globulins can be fractionated into β-vignin, α-vignin and γ-vignin. *Vigna unguiculata* protein is rich in the amino acids, lysine and tryptophan (Lim, 2012). These proteins have an isoelectric point of approximately 4.5, thus *Vigna unguiculata* protein is negatively charged at a neutral pH (Abdel-Shafi, et al., 2019).

Performance in water treatment

In a study by Mbogo (2008), the active components were aqueously extracted from *Vigna unguiculata* seeds. The coagulant product was able to remove 99.2% turbidity from a sample with an initial turbidity of 482 NTU. The resulting low residual turbidity of 3.67 NTU was achieved at an optimal coagulant dose of 800mg/l. When the natural coagulant was used in combination with aluminium sulphate (80% natural coagulant and 20% aluminium sulphate), 100% turbidity removal was achieved at the same coagulant dosage of 800mg/l.

In a study by Sotheeswaran, et al. (2011), *Vigna unguiculata* active coagulants were aqueously extracted and applied in turbidity testing processes. This natural coagulant was able to reduce turbidity from 250 NTU to 100 NTU over a 24-hour period, thereby outperforming *Vigna mungo* and *Arachis hypogaea. Vigna unguiculata* was also able to reduce the total hardness of water by 24%.

A study was conducted to determine the effectiveness of *Vigna unguiculata* as a natural coagulant for the treatment of synthetic turbid water. Active components were aqueously extracted from the seed and were used in jar tests to establish optimum conditions for coagulation. Results show that from initial turbidities of 250 NTU and 400 NTU, *Vigna unguiculata* was able to reduce turbidity by 72% at an optimum dose of 4ml and by 45% at an optimum dose of 0.5ml respectively. In an antimicrobial study, *Vigna unguiculata* was capable of flocculating, aggregating and inhibiting the growth of bacteria (Blix, 2011).

A study was conducted to determine the effectiveness of *Vigna unguiculata* as a natural coagulant for turbidity removal (Hussain & Haydar, 2019). In order to optimise the coagulation process, pH and dose was varied from 2-10 and 20-100mg/l respectively. Results showed that at a pH of 4 and dose of 20mg/l, 97.6% turbidity reduction from an initial turbidity of 200 NTU was achieved. However, *Vigna unguiculata* can be used for water treatment with little to no pH adjustment as it can achieve >95% turbidity removal in the pH range of 4-6 at relatively low doses of 20-40mg/l.

The optimum dose for maximum coagulation activity was investigated by Marobhe, et al. (2007). In this study, it was determined that after 90 minutes of settling, aqueously extracted *Vigna unguiculata* coagulant at a dose of 12.5μg/ml was able to achieve 80% coagulation efficiency. Maximum coagulation activity was attained at dosages of 1μg/ml and 2μg/ml when active components were salt extracted using 0.3M and 0.6M NaCl solutions respectively. The effects of floc settling time was also carried out and it was determined that 92% turbidity removal was achieved after 2 hours of settling, when active coagulants were salt extracted using 0.6M NaCl. When proteins were extracted using 0.3M NaCl, 60% and 89% turbidity removal could be achieved at settling times of 60 minutes and 2.5 hours respectively. The study also concluded that *Vigna unguiculata* active components extracted using 0.6M NaCl were more heat resistant than those extracted at 0.3M NaCl, making heat treatment a viable option for the extraction and handling of seed extracts. Further, *Vigna unguiculata* performed well in a pH range of 5.5 to 8.5 but performed optimally at a pH of 7.5, where 95% coagulation activity was observed after 2 hours of settling (Marobhe, et al., 2007).

The findings of Ahmed, et al. (2012) indicated that the proteins present in *Vigna unguiculata* become increasingly soluble in solution with increasing salt concentration up to a salt concentration of 0.4M. Thus, the optimum salting-in concentration for this study was determined as 0.4M.

Vigna mungo

Background

Vigna mungo (commonly referred to as black gram or Urad bean) belongs to the Fabaceae family as well as the Leguminosae family. The plant is indigenous to India where it is a highly prized pulse and has been cultivated from ancient times. Traditionally, the crop is cultivated in South Asia and surrounding regions such as Pakistan, Bangladesh, Myanmar and Afghanistan. *Vigna mungo* is drought tolerant and can grow in dry areas and in a variety of soils. However, this plant grows optimally in a temperature range of 27-30 ˚C and in loamy soils (Lim, 2012).

Vigna mungo is an erect to sub-erect annual plant that grows 30-100cm high (shown in Figure 18). Leaves trifoliate and are 4-10cm by 2.5-5cm. The flowers are bright yellow, small and axillary. The fruits are upright, cylindrical pods that are 4-6cm long and have hooked beaks (Lim, 2012).

Vigna mungo pods contain 4 to 10 seeds which are ellipsoidal, grey-black to black in colour and have a white hilum protruding from the seeds (Lim, 2012). The seeds of *Vigna mungo* are depicted in Figure 19. *Vigna mungo* global production was 3.2 million tons in 2018, with India contributing to more than 70% of the world *Vigna mungo* production (Research and Markets, 2019).

Figure 18: *Vigna mungo* plant (Art Fire, 2020)

Figure 19: *Vigna mungo* seeds (Art Fire, 2020)

Uses and advantages

Vigna mungo seeds are cooked and eaten whole or utilised as a pulse. When the seeds are dehusked, it can be used in the preparation of idli, dosa, papadum and cooked dhal. The use of the plant in food processing relies on its functional properties (such as foaming, texture, gelation, emulsification, and oil and water absorption capacity) that affect food quality and processing applications (Wani, et al., 2013).

Vigna mungo provides excellent nutritional benefits as it is high in protein, carbohydrates, fibre, amino acids, folate, niacin and minerals like iron, potassium, phosphorous, magnesium, calcium, and zinc. Further, *Vigna mungo* is rich in flavonoids which have valuable antioxidant properties. *Vigna mungo* also showcased immunostimulatory activity and was effective in reducing cholesterol levels. In traditional medicine, *Vigna mungo* was utilised in the treatment of diabetes, inflammation, dyspepsia, and anorexia. Industrially, *Vigna mungo* is used for silage, forage, hay and chicken pasture (Lim, 2012).

Protein structure

Vigna mungo contains 24.5-28.4% proteins, 51.5-63.3% carbohydrates, and 1.1-1.4% fat (Wani, et al., 2015). Solubilised seed proteins constitute 81% globulins, 13% albumins, 2% glutelins and 4% prolamins (Padhye, 1978). 82% of the *Vigna mungo* globulins are made up of major protein subunits with molecular weights of 140kDa and 55kDa. The major protein subunits of *Vigna mungo* albumins have molecular weights of 140kDa, 84kDa, 56kDa and 39kDa (Padhye & Salunkhe, 1979). The isoelectric point of *Vigna mungo* lies between 4 and 5 therefore it is negatively charged at a neutral pH (Kamani, et al., 2019).

Dehulled and defatted *Vigna mungo* flour contains approximately 25% proteins, which are composed majorly of globulins (63%), as well as albumins (12%), glutelins (21%) and trace amounts of prolamins. The globulins present in *Vigna mungo* are legumin and vicilin. Amino acid analysis showed that glutamic acid, aspartic acid and lysine were present in the seed (Lim, 2012).

Performance in water treatment

Vigna mungo was tested as a natural coagulant in a study by Mbogo (2008). The aqueously extracted coagulant was able to reduce the turbidity from 482 NTU to 0 NTU (100% turbidity removal) at an optimum dose of 800 NTU. Even at a dose of 600mg/l, the turbidity was reduced to an acceptable level of 2.9 NTU. Aluminium sulphate was able to achieve 100% turbidity removal at a higher dose of 1000mg/l; therefore, *Vigna Mungo* outperformed the conventional coagulating agent. When used as a coagulant aid (80% natural coagulant and 20% aluminium sulphate), 100% turbidity removal could be achieved at a lower dose of 600mg/l. Solar radiation used in this study, to disinfect the water treated by *Vigna Mungo,* was able to remove 100% of *Bacillus spp.* and *Enterobacter spp*. or *Pseudomonas* bacteria.

Aqueous extraction of *Vigna Mungo* seed protein was able to produce a protein isolate yield of 18.6%-19.8% in a study by Wani, et al. (2015). *Vigna Mungo* active coagulating agents extracted by aqueous extraction were able to reduce turbidity from 250 NTU to 140 NTU over a 24-hour period, thereby outperforming *Arachis hypogaea* (Sotheeswaran, et al., 2011). Sotheeswaran, et al. (2011) also found that *Vigna Mungo* showcased a hardness removal efficiency of 24%.

Vigna mungo was tested as a natural coagulant to treat raw water samples at different initial turbidity levels of 100 NTU, 250 NTU, and 500 NTU. At an initial turbidity level of 500 NTU, the natural coagulant derived, via salt-extraction (0.5M NaCl concentration), from *Vigna mungo* seeds, was able to reduce turbidity by 75.1% at an optimal dosage of 0.4mg/l. At the same dose, the natural coagulant was able to reduce turbidity by 71.6% and 75.7% in raw water with initial turbidities of 250 NTU and 500 NTU respectively (Sasikala & Muthuraman, 2017).

2.3.1.2.3. Summary of key coagulants

Table 3 provides a summary of the advantages and disadvantages of inorganic coagulants conventionally applied in water treatment and natural organic coagulants that are currently being investigated for use in front-end water treatment. The investigation into alternative coagulants (as opposed to inorganic coagulants) for front-end water treatment was majorly substantiated by the high costs, high sludge volume produced, and the health risks associated with inorganic coagulants. Organic coagulants such as *Moringa oleifera,* were favoured for use in this application for their effectiveness in turbidity removal as well as their ability to mitigate the negative economic, health and environmental impacts that coagulants such as aluminium sulphate has been criticised for.

However, organic coagulants, especially legumes, pose the risk of low availability due their seasonal variation in growth and their current widespread use as food sources. In addition, there may be challenges experienced in the recovery and loading of plant proteins based on their unique protein properties. If plant proteins exhibit low extractability and loading capacity, then high doses of coagulant will be required to facilitate effective turbidity removal.

Table 3: Summary of the advantages and disadvantages of inorganic and organic coagulants

<table>
<tr><th colspan="2">Types of coagulants</th><th>Advantages</th><th>Disadvantages</th></tr>
<tr><td rowspan="2">Inorganic coagulants</td><td>Aluminium sulphate</td><td>-Effective in reducing the turbidity, organic matter and microorganism content in water
-Readily available since it does not depend on seasonal variation</td><td>-High sludge volume produced
-Associated with health risks to humans
-Toxic to aquatic life
-Higher cost than plant coagulants
-Requires pH adjustment</td></tr>
<tr><td>Ferric chloride</td><td>-Effective coagulant in reducing turbidity
-Performs well over wide pH and temperature range
-Better floc settling characteristics than aluminium sulphate
-Readily available since it does not depend on seasonal variation</td><td>-Increases corrosivity of water
-Water requires further specialised treatment to remove stains
-Higher cost than plant coagulants</td></tr>
<tr><td rowspan="5">Organic coagulants</td><td>Moringa oleifera</td><td rowspan="5">-Effective coagulant in turbidity removal
-Lower sludge volume produced compared to inorganic coagulants
-Lower cost than inorganic coagulants
-Biodegradable
-Does not require pH adjustment or consume alkalinity
-Short growing period
-Easy cultivation
-Currently consumed by human beings and was found to pose no immediate risk to human health</td><td>-Lack of conclusive research into toxicity
-Competition for use as a food source
-Seasonal variation may impact availability
-Protein extractability and loading challenges</td></tr>
<tr><td>Glycine max</td><td rowspan="4">-Competition for use as a food source
-Seasonal variation may impact availability
-Protein extractability and loading challenges</td></tr>
<tr><td>Cicer arietinum</td></tr>
<tr><td>Vigna unguiculata</td></tr>
<tr><td>Vigna mungo</td></tr>
</table>

2.3.2. Flocculation

In the coagulation process, colloidal particles are neutralised and form microflocs due to the addition of a suitable coagulant. If the coagulant process is not efficient i.e. it does not take place under near optimal conditions, then the resulting microflocs will be very small or fragile, which will affect the settling rates of these flocs in separation processes. Therefore, flocculation is employed to agglomerate slow-settling and non-settleable microflocs into larger and more dense flocs that can be more easily removed in later treatment processes (Lee, et al., 2012). Flocculation can occur via two routes: micro-flocculation (or perikinetic flocculation) and macro-flocculation (or orthokinetic flocculation). Micro-flocculation encompasses the agglomeration of particles due to the thermal motion of the fluid molecules, while macro-flocculation involves applying a velocity gradient, through mixing, to aggregate the particles (Tzoupanous & Zouboulis, 2008).

Water treatment plants opt for one of two methods, namely coagulation-flocculation or direct flocculation. Coagulation-flocculation is the use of the coagulants to form microflocs and thereafter the use of flocculants to agglomerate these flocs. The coagulation process takes place over a short period of time, in some cases seconds, whereas flocculation takes place over a duration of 20-40 minutes. Direct flocculation is used as an alternative to the coagulation-flocculation process in order to reduce costs. This direct route typically uses a cationic polymer which serves to neutralise the negatively charged colloids and aggregate the destabilised particles to form flocs. Direct flocculation renders the use of coagulants redundant, which reduces sludge volumes and makes pH adjustment unnecessary, thereby reducing operational costs. However, water that contains a high level of soluble solids and inorganic substances can only be treated effectively through the use of the coagulation-flocculation process. Therefore, the method of achieving settleable flocs highly depends on the characteristics of the water to be treated (Chong, 2012).

The majority of flocculants used in wastewater treatment can be generally categorised as synthetic polymers (mostly polyelectrolytes) or biopolymers. Commercially used synthetic flocculants include polyacrylamide, polyamine, polyacrylic acid and poly (diallyl dimethyl ammonium chloride). These synthetic polymers are favourable for their high efficiency at low doses, for their ability to form large, dense flocs that settle easily and for their low overall cost. However, these synthetic flocculants have been associated with having a high toxicity and negative environmental impact (as they have slow biodegradability and the degradable products are potentially carcinogenic). For these reasons, the use of bio-flocculants, such as chitosan, tannins and cellulose, are becoming more prevalent in water treatment due to their non-toxicity, biodegradability, ease of availability and low associated costs (Lee, et al., 2014).

2.3.3. Sedimentation

Sedimentation is a process whereby particles in suspension settle to the floor of the water body by the action of gravity. The settled particles, which are inorganic or organic, are referred to as sediment, or sludge (in water treatment). Sediment can be classified as either fine sediment or coarse sediment. Solid particles (such as sand, silt and clay) which have particle sizes less than 2mm are called fine sediment. Coarse sediments are those sediment particles that have a particle size greater than 2mm and include cobble, gravel and boulders (Amini, 2018). In a natural setting, these particles may arise due to the action of soil erosion where particles are deposited into water bodies like rivers, reservoirs and wetlands. Sedimentation in these instances, not only degrades the water quality, but also reduces the capacity and lifespan of the water body (Tundu, et al., 2018)

Particles generally settle in a dispersed or flocculated form, where the settling process of particles depends generally on the ionic strength, pH, and solid-to-water ratio within a water body. Dispersed settling usually occurs naturally, without any pre-treatment, and encompasses particles spreading out and settling without interacting with each other. In this case, the coarser particles settle at the bottom and the finer particles settle at the top. Flocculated settling involves flocs, from flocculation processes, settling with a uniform particle distribution throughout the water body. Particles can settle interchangeably by dispersed and flocculated settling, therefore the factors affecting settling must be carefully controlled (Kaya, et al., 2006).

In water treatment, plain sedimentation can take place whereby water is stored undisturbed in a settling tank for a period of time to allow the particles to sediment by the action of gravity. Thereafter, careful removal of the settled water can be achieved by decanting or ladling. This simplistic method of separating out settleable solids is favoured for its low-cost and ease of application (WHO, 2002).

Sedimentation can take place before coagulation to reduce the concentration of particles in suspension, particularly grit or coarse matter, and thereby reduce the amount of coagulant required (IWA Publishing, 2020). However, sedimentation usually takes place after coagulation-flocculation processes to remove finer silt and clay particles that cannot be completely removed via coagulation-flocculation processes (WHO, 1997).

Plain settling can be accomplished using a plain sedimentation tank (depicted in Figure 20). The plain sedimentation tank can be used to reduce the amount of coarse and fine sediment present in a water body. The turbid water is passed through the inlet, allowed time to settle, and the resulting water is removed at the outlet. Baffles are included in sedimentation tanks to enable a regular flow pattern throughout the tank (WHO, 1997).

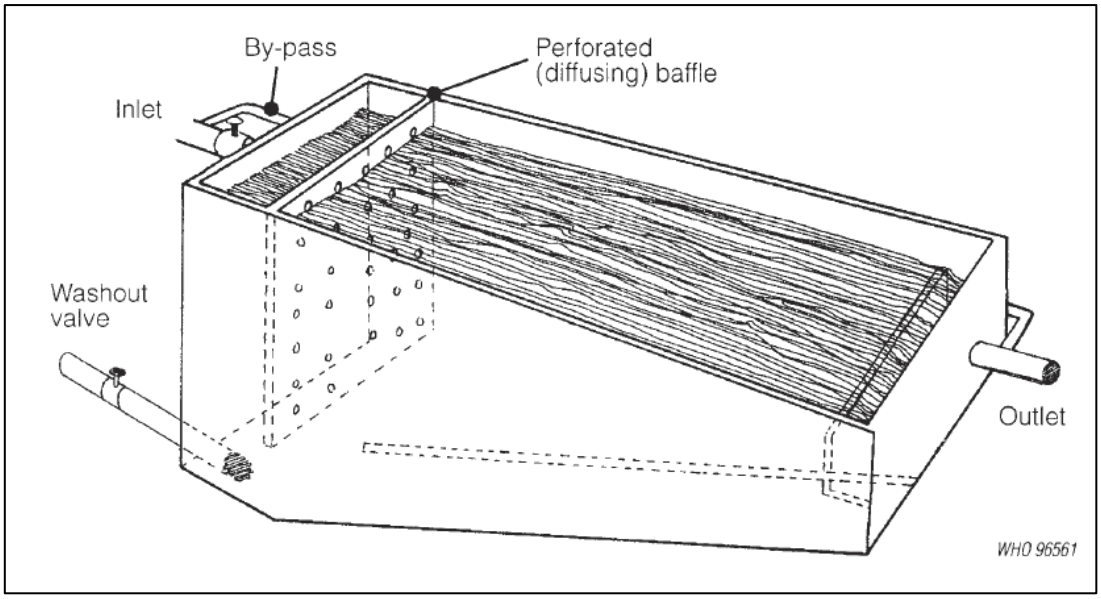


Figure 20: Plain Sedimentation Tank (WHO, 1997)

2.3.4. Filtration

The filtration of water involves the removal of particulate matter (such as suspended particles, various micro-organisms, and chemical contaminants) in order to produce clean and safe water (Mao, 2016). In a natural process, suspended materials can be removed from groundwater as the fluid percolates through soil. In water treatment processes, filtration usually involves passing a suspension through a porous medium (known as a filter medium) which results in solid particles (usually floc) being retained on top of the medium or within the pores of the medium (called the retentate), and the discharged fluid(called the filtrate) flowing through the medium.

Filtration is generally affected by the pressure differential across the filter medium and the temperature of the suspension. A pressure gradient is important to ensure that the solvent is forced through the filter medium while the particles are retained (Makino, 2018). The temperature of the suspension has to be carefully controlled as it affected the liquid phase viscosity which consequently influences the ability of the liquid to flow through the filter medium.

Different filter medium can be used depending on the filtration technique; these include, sand, wool or cotton fabric, wire cloth, coal, and metal powder (Cheremisinoff, 2002). Filter aids, such as diatomaceous earth or carbon, can be used to improve filtration efficiency and protect the filter medium during filtration. It may be necessary to wash the filter cake in order to ensure a clean separation of the mother liquor and the solids. Further, dewatering may be needed, whereby a clean fluid is passed through the filter cake to recover any liquid that may have been retained during the filtering or washing process.

Filters are generally classified as pressure filters or gravity filters. Pressure filters use the force of pressure to move the water through the filter medium. Gravity filters (such as slow sand filters and rapid sand filters) are more commonly applied and use the force of gravity to propagate water through the filter. Slow sand filters, as shown in Figure 21 contain sand and a biologically active layer that serves to not only filter but chemically treat contaminated water. The slow movement of the fluid through the filter aids the development of the biological layer. Rapid sand filters, shown in Figure 22, use coarser sand than slow sand filters, and rapidly trap suspended materials using the filter sand (without the use of the biological layer) (Pizzi, 2010).

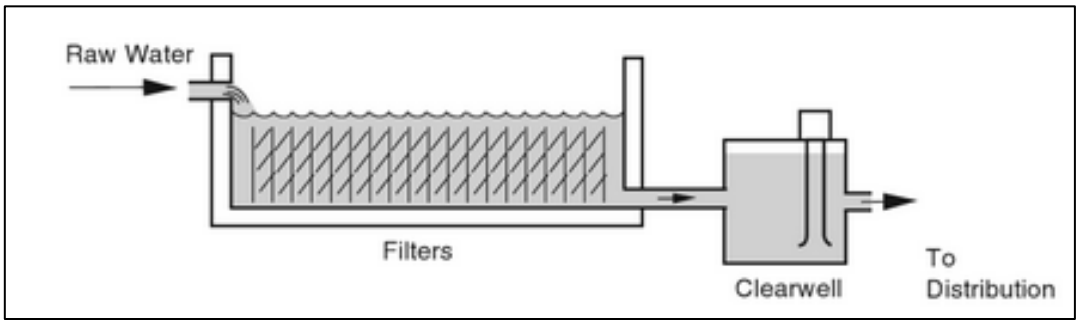


Figure 21: Slow sand filter plant (Pizzi, 2010)

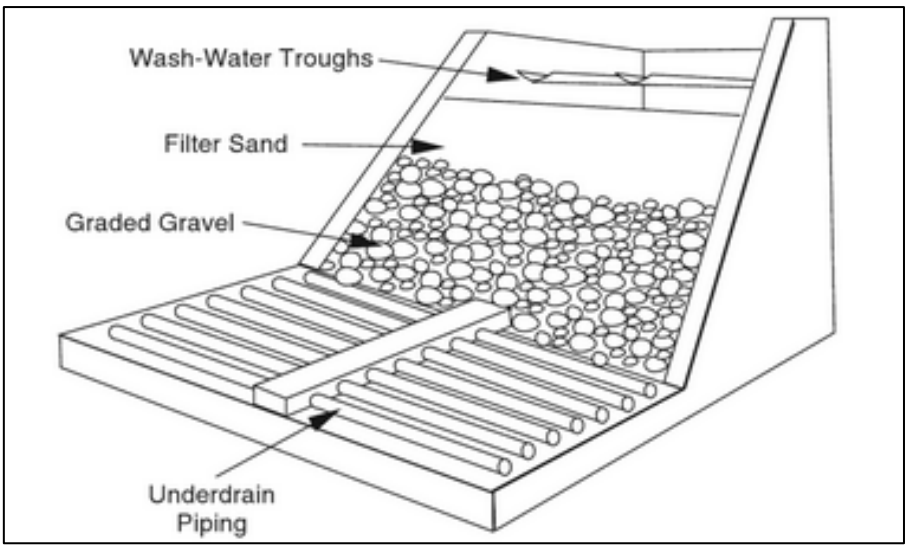


Figure 22: Rapid sand filtration system (Pizzi, 2010)

In conventional water treatment processes, the filtration step comes after the sedimentation process and before the disinfection stage. After the sedimentation step, water with a turbidity in the range of 2 NTU to 3 NTU is filtered as a polishing step, prior to disinfection. Turbidity greatly affects processes like disinfection as the suspended particles shield microorganisms in suspension from the disinfectants or they chemically combine with the disinfectants and reduce its effectiveness in killing microorganisms. Thus, the filtration process is vital to reducing turbidity to an acceptable level such that the disinfection process can be carried out effectively (Pizzi, 2010).

2.3.5. Disinfection

Disinfection refers to the treatment process used to destroy pathogens such as bacteria, viruses, and fungi. Although many pathogens are destroyed in the earlier stages of the conventional water treatment process, disinfection is still necessary to ensure that pathogen loading is reduced to an acceptable level. This is important to prevent the transmission of diseases such as typhoid fever, salmonellosis, shigellosis and cholera.

A significant concentration of flocs, and other suspended particles, protect pathogens from the action of disinfectants, stimulate bacterial growth and result in an immense disinfectant demand (WHO, 2008). It is thus imperative to reduce turbidity levels significantly prior to disinfection. This is usually accomplished through conventional water treatment processes such as coagulation, flocculation, sedimentation and filtration.

Disinfection can be attained through physical and chemical processes. The choice of the disinfection method depends on the efficacy against pathogens, aesthetic quality of the treated water, availability and cost of the treatment system, and finally the health and environmental impacts (Pandit & Kumar, 2013).

Physical processes include heat treatment and ultraviolet radiation treatment. Heat treatment is a simple process that involves boiling water for at least 5 minutes to ensure that organisms present in water are destroyed. This is usually carried out for small quantities of water since the energy requirement for large-scale application is not feasible. Ultraviolet radiation is a potent method of inactivating pathogens such as *Cryptosporidium* and *Giardia* by affecting the genetic material of the cell. This is an effective, quick method but may require supplementary disinfection, such as chlorination, to have a residual effect (Pizzi, 2010).

Chemical processes include treatment using disinfectants such as ozone, halogens (such as chlorine), hypothalous salts, and enzymes. In addition to destroying pathogens, these chemicals control biological growth, tastes and odour, and aid flocculation. Pathogens can be removed through the use of chemical disinfectants like chlorine, ozone and ultraviolet light. However, the formation of disinfection by-products (such as trihalomethanes) can occur due to the reaction of these disinfectants with naturally occurring substances in the water like organic matter, certain contaminants, and bromide (Benson, et al., 2017). These disinfection by-products have been associated with having carcinogenic, cytotoxic, genotoxic, and mutagenic effects (Plewa, et al., 2010; Richardson, et al., 2007). Thus, in order to prevent or reduce the formation of disinfection by-products, it is imperative to either carefully control doses of chemical disinfectants, use chemical disinfectants that are less prone to forming by-products, use non-chemical disinfectants or remove the disinfection by-products after disinfection treatment (WHO, 2008).

2.3.6. Testing effectiveness of water treatment for turbidity reduction

The following factors affect the efficiency of the coagulation-flocculation process: type, feed concentration and dosage of coagulant, pH, sequence of chemical addition, degree and duration of mixing and the velocity gradients applied during flocculation (Bratby, 2016). Prior to carrying out any tests of the coagulation-flocculation efficiency, it is advisable to analyse the water source (especially if obtained from natural sources) for prominent contaminants that could significantly impact process efficiency.

Conventional jar tests can be utilised to simulate the coagulation-flocculation process on a lab-scale, in order to establish optimal process conditions, in a simple, fast and economical manner. The jar test firstly involves the addition of coagulants to jars of water. The contents of each jar are then stirred to enable the formation and settling of flocs. Thereafter, samples are drawn from each jar and an analysis of the effects of different variables (such as dose and pH) on process efficiency is undertaken. It is imperative that jar tests be carried out regularly, especially if there are changes to process conditions (such as the type or dose of coagulant) to ensure efficiency and adherence to general water quality standards (Satterfield, 2005).

The jar test apparatus should ideally consist of a set of jars with a rack of stirrers as in Figure 23. The stirrers should be operated by a single motor and have variable speeds to carry out slow and rapid mixing for the coagulation-flocculation process to occur. Jars with a large capacity should be employed for ease of chemical addition, and since the jar surface is used as an adsorption site for coagulants (Bratby, 2016)

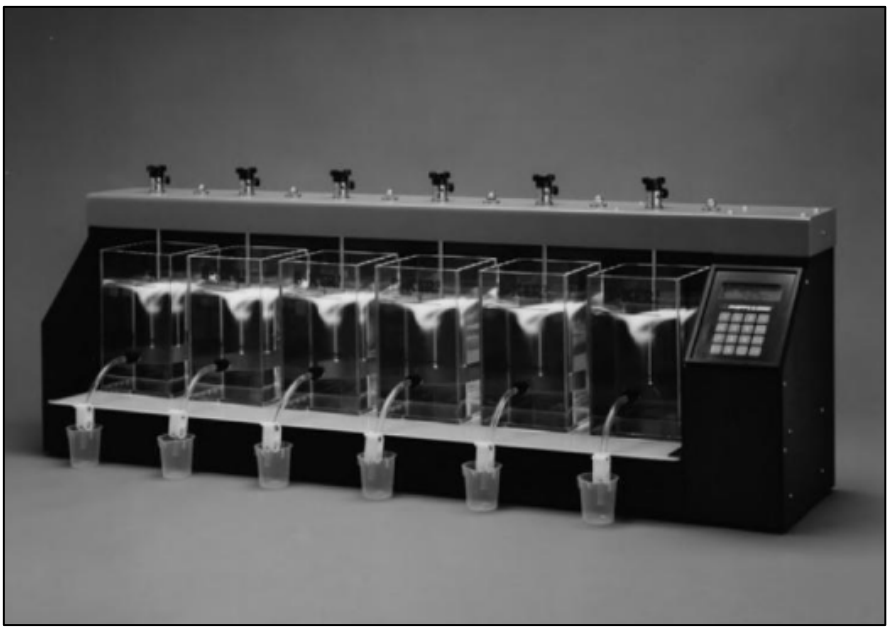


Figure 23: Jar test apparatus (Bratby, 2016)

2.4. Protein recovery

Proteins are macromolecules that primarily contain carbon, hydrogen, oxygen, nitrogen and sulphur, and are made up of structural units called amino acids. Proteins can be differentiated by the type and number of amino acids that make up the protein. The function of protein is to transport material within a cell and across membranes, organise DNA, and catalyse chemical reactions (Blanco & Blanco, 2017). Proteins are an important food source as they contain vital amino acids that are required to meet human physiological needs. Further, proteins have excellent medicinal properties making them useful in medical applications (Nehete, et al., 2013).

The choice of protein for study usually depends primarily on the convenience of growing. Animals typically used for protein study include rats, rabbits, cows and pigs. Animal protein generally contains high quantities of fat which could potentially lead to diseases such as heart disease and high blood pressure. Plant studies are considerably less prevalent, compared to animal studies, due challenges such as seasonal variations in plants. Vegetables, legumes and fruits are excellent sources of proteins. Commonly studied plants include soybean, wheat, pea, sunflower and rice, which are favoured for their relatively high protein content and growth patterns (Scopes, 1994).

Typically reasons why proteins recovery methods are generally employed are as follows (Grandison & Lewis, 2006).

- To recover the protein from food to improve their functional properties
- To separate and remove proteins from toxic substances in food
- To recover biologically active proteins like enzymes and hormones
- To fractionate proteins (in some cases using solubility characteristics)

Protein solubility properties make them favourable for use in the water treatment industry. These solubility properties include the ability to form colloidal solutions in water, the dependence on electrostatic charges (which depends on amino acid properties and the pH of the solvent), and the dependence on the isoelectric point (which typically ranges from 5 to 8.5) (Nehete, et al., 2013).

Legumes typically have a high protein content. This protein is stored in seed organelles as a source of nutrition for the seeds to promote growth and development. These proteins, called storage proteins, are classified according to their solubility properties. The water-soluble proteins are albumins, while globulins are soluble in dilute salt solutions. Glutelins are soluble in dilute acid or alkali and prolamins are soluble in alcohol-water mixtures (Kristianto, 2017). The major storage proteins however are globulins and albumins, where globulins represent approximately 70% of the total protein content.

Globulin proteins can be classified as 11S(legumins) or 7S(vicilins) proteins. The vicilin protein is trimeric and has a molecular weight of 150-800kDa, while legumin protein is hexameric and has an overall molecular weight of 300-400kDA. The hydrophobic sections of these proteins are found buried at the interior while the hydrophilic sections are found at the protein surface (Karaca, et al., 2011).7S proteins can be further classified as β-conglycinin, γ- conglycinin and the basic 7S globulin. 11S proteins consists of five subunits: $A_{1a}B_{1b}(G_1)$, $A_2B_{1a}(G_2)$, $A_{1b}B_2(G_3)$,$A_5A_4B_3(G_4)$, and $A_3B_4(G_5)$ (Yuan, et al., 2002). Albumin proteins make up approximately 10-20% of the total protein content in legume seeds and have a wide range of molecular weights(16-483kDa) (Karaca, et al., 2011).

2.4.1. Preparation of plants for protein recovery

In order to obtain the active components from the plant seeds for water treatment, preparation steps need to take place. There are several techniques to disrupt organism cells and enable easy release of the proteins into solution. These techniques include using a hand-operated or motor-driven glass homogenizer, waring blade-blender(or food processor), vibrating glass bead mill, Manton-Gaulin cell disintegrator and ultrasonic probe (Scopes, 1994).

Legumes are typically prepared by firstly manually removing the hull (which in some cases may require prior soaking of the plant) and splitting the seed. In general, the removal of the hull leads to a reduction in the content of fibre and tannin, and improves appearance, texture, palatability and digestibility (Grandison & Lewis, 2006).

Screening is also used to remove contaminants that are considerably larger or smaller than the desired components (Bichi, et al., 2012), and is used to obtain desired particle sizes(typically <600μm) for water treatment (Asrafuzzaman, et al., 2011). In industrial processes, magnetic separators and electrostatic separators remove contaminants by taking advantage of the difference in magnetic and electrostatic charge respectively. Further, wet processing methods to clean samples can be used. These techniques include soaking with agitation in water, using high-pressure spraying or using flotation. It must be noted that water has the ability to separate proteins by reducing interparticle adhesion, therefore pre-soaking plant materials in water has the added benefit of improving separation. However, there are high costs involved in adding water and thereafter dewatering (Grandison & Lewis, 2006).

2.4.2. Extraction of plant proteins

Plant extracts have been extensively used in many industries such as the pharmaceutical industry, cosmetic industry and nutraceutical industry. In order to obtain useful compounds from plant materials, suitable extraction and purification techniques need to be applied. Several methods have been utilised to carry out the extraction of active components from plant material in order to improve its coagulation activity.

Conventionally applied extraction methods include: normal aqueous extraction (using distilled water), salt extraction, oil removal then aqueous extraction, oil removal then salt extraction, oil removal then microfiltration and finally oil removal followed by both salt extraction and microfiltration (Bichi, et al., 2012).Selection of an extraction technique is highly dependent on the type of application, economic factors as well as health and environmental impacts.

2.4.2.1. Aqueous extraction of plant proteins

Conventional extraction techniques involving plant materials include: hydrodistillation, heating-reflux extraction and maceration. However, these methods have been found to have high energy and time consumptions, low efficiency and high associated costs (Reinoso, et al., 2017). Further, due to the toxicological effects of solvents conventionally used in solid-liquid extraction, research has been focused on developing a safer and more environmentally friendly alternative. In addition, there has been an increasing awareness of the importance of quality protein for human nutrition, which thereby motivated the improvement of protein acquisition processes and practices.

Aqueous extraction has been utilised in the removal of oil and protein from oilseed materials. The process has been applied to coconuts and peanuts where 91% and 92% protein recovery was achieved respectively. Aqueous extraction offers various industrial benefits such as simplicity(since there are fewer processing steps than conventional extraction), low initial capital investments, capability of discontinuous operation, safe operation (due to low operational danger and air pollution from solvent loss), production of different products, and capability to use chemicals to remove undesired substances. However, aqueous extraction has disadvantages such as relatively low extraction efficiency, the production of protein products with a high oil content which may result in storage stability issues, and a high potential for microbial contamination due to the increased number of processing steps that the material has to go through while wet (Cater, et al., 1974).

Protein and oil can be recovered simultaneously from plant material. The protein can be recovered in the solids or aqueous phase based on the operating conditions. Parameters in the unit operations vary slightly based on the plant material and are governed by differences in physical structure and chemical composition of the plant materials. Efficiency of extraction greatly depends on the unit operations used to process the plant material which are typically, comminution, agitation, solid-liquid separation through centrifugation, and drying of the final products (Cater, et al., 1974).

In aqueous extraction of proteins from plant materials, comminution firstly takes place (typically via grinding) to break cell walls so that the protein can be easily exposed and released (Rosenthal, et al., 1996). Wet or dry grinding can be employed in this step.

While dry grinding of plant material solely involves size reduction, wet grinding physically and chemically alters the material being ground (Solanki, et al., 2005). The choice of wet or dry grinding depends on the seeds' initial moisture content, physical structure as well as its chemical composition (Cater, et al., 1974)

The next step in aqueous extraction involves the comminuted extracts being dispersed in the extractant. Agitation is generally used to enhance the dispersion and extraction. In this step, the soluble components of the plant constituents (the protein) diffuse in the extractant while other insoluble components (such as plant oil) remain out of solution. Centrifugation thereafter takes place to separate the aqueous extract into solid and aqueous phases. Most of the proteins would be recovered in the solid phase. The remaining solids in the aqueous phase can be recovered by isoelectric precipitation. It must be noted that solid-to-water ratio, pH, time, temperature, particle size and degree of agitation affects extraction efficiency and must be carefully monitored and controlled (Rosenthal, et al., 1996).Simplified methods such as mixing the powder in water and filtering using muslin cloth (Muyibi & Evison, 1995) or Whatman filter paper (Choudhary & Neogi, 2017) can also be commonly applied as a low-cost alternative.

Water is low-cost, non-toxic, non-flammable, environmentally benign, and enables clean processing with minimal pollution (Petigny, et al., 2015). For these reasons, water is commonly used as a protein extractant in aqueous extraction after pH adjustment, to ensure that it is not near the isoelectric point of proteins (since aqueous extraction operates based on the solubility characteristics of proteins).

Water has been extensively applied in the extraction of biologically active compounds. Many different methods of extraction using water have been employed to recover active components from raw materials. These methods include decoction, infusion, subcritical water extraction, hydrodistillation, ultrasound, microwave and enzyme extractions using water. However, water does have some limitations in its use as an extractant such as its low solubility of non-polar compounds and energetic requirements to concentrate products (Mihaylova & Lante, 2019).

2.4.2.2. Salt extraction of plant proteins (salting-in and salting-out)

The salting-in phenomenon states that when the salt concentration of a solution, containing a protein source, is increased, the solubility of the proteins will increase, and the proteins will be extracted into solution from the protein source. Increasing the salt concentration beyond the point at which extractability is maximised will result in the salting-out phenomenon. The salting-out phenomenon states that increasing the salt concentration beyond the optimum point for extraction leads to a decrease in protein solubility and subsequent precipitation of the extracted proteins out of solution.

Salt extraction of proteins from various sources (such as plant seeds) employ the principles of salting-in and salting-out to achieve high extraction efficiencies. This method of extraction has been commonly applied in the recovery of proteins due to its high ionic strength. Effective salts that can be used for this purpose include sodium chloride, potassium chloride, lithium chloride, divalent magnesium and calcium chloride. A salt extraction of *Moringa oleifera* coagulant protein study by Jain, et al. (2019), found that divalent cationic salts (such as $MgCl_2$ and $CaCl_2$) showcased higher protein extractability than monovalent salts (such as NaCl and KCl), however the proteins extracted by divalent salts could not be precipitated upon watering-out. This was due to the high ionic strength of divalent salts which required more dilution to reduce the ionic strength for precipitation. By increasing dilution however, the distance between hydrophobic groups increases and the balance of hydrophilic-hydrophobic forces becomes affected which consequently affects precipitation (Jain, et al., 2019). Therefore, monovalent salts are typically preferred for the ease at which proteins can be precipitated.

The use of salts, particularly sodium chloride (NaCl) not only aids extraction, but adds desirable flavour, offers the benefit of antimicrobial properties and improves the water holding capacity of proteins. The chloride ion operates by causing protein swelling and disruption to the protein structure which in turns increases protein solubility and its water holding capacity (Gordon & Barbut, 1992).

Figure 24 shows the effect of salt concentration on protein solubility. At low ionic strengths, the addition of salts, such as sodium chloride, increases protein solubility by a process of salting-in (Maurer, et al., 2011). With an increase in ionic strength, an isoelectric point is reached where minimum protein solubility occurs. Beyond the isoelectric point, protein solubility decreases as a function of ionic strength and this is classified as the salting-out region (Maurer, et al., 2011).

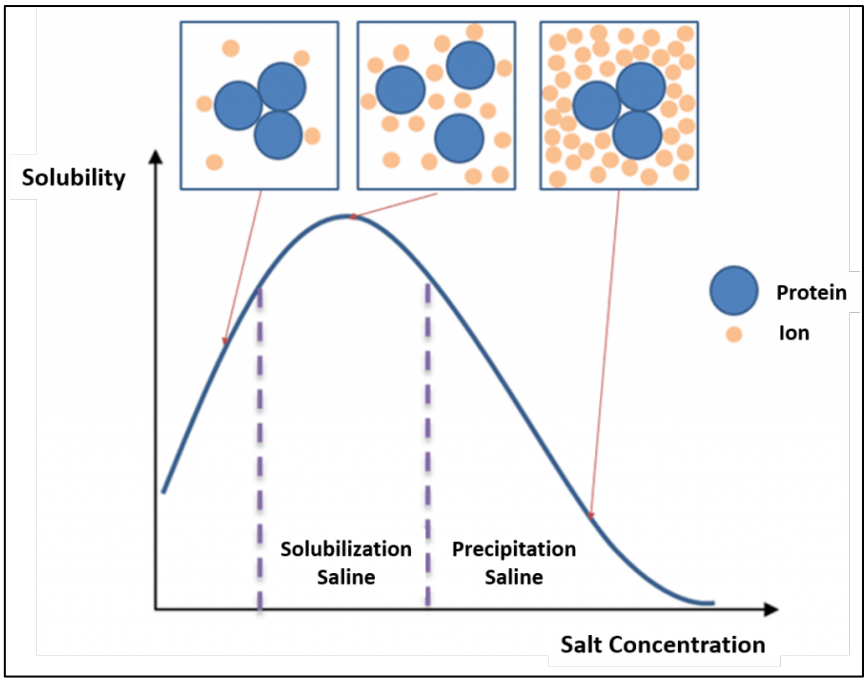


Figure 24: Effects of salt concentration on solubility (Western Oregon University, 2020)

The process of salting-in is due to the salt molecules, at low concentrations, stabilising the proteins and other polyelectrolytes by electrostatic interactions (that depends of the medium ionic strength); this in turn increases the protein solubility. At high concentrations of salt, stabilisation or denaturation of proteins and salting-in or salting-out can occur depending on the salt nature and concentration (Arakawa & Timasheff, 1984).

Salting-out is a useful technique in protein purification as a specific salt concentration can precipitate and remove undesired proteins and a different salt concentration can precipitate the desired protein. This method is favoured for its simplicity of application. Further, the precipitated protein produced by salting-out is highly stable, therefore, the process can be used to improve the shelf-life of the protein (Lee, 2017).

When the salting-out effect occurs, the protein precipitates because at high salt concentrations, the salt competes with the protein in binding to the water, and the reduced protein-water interactions then leads to increased hydrophobic interactions between the protein molecules. Salts that increase the surface tension of water the most are preferred to carry out salting-out. Ammonium sulphate is a favoured reagent for this purpose, due to its relatively high solubility compared to other phosphate salts, its low cost, its availability as well as its ability to inhibit bacterial growth (Wingfield, 2001). After the salting-out process, the sample would contain a high concentration of ammonium sulphate and thus must be purified further by methods such as hydrophobic interaction chromatography (HIC) (Doung-Ly & Gabelli, 2014).

2.4.3. Fractionation of proteins

Protein solubility is due to the hydrophilic amino acid side chains that interact with water. When a compound is added to the solution, it may interfere with the protein-water interaction by interacting with the water itself and reducing the solubility of the protein. Since the protein-water interactions occur less, the protein-protein interactions take precedence and the protein begins to aggregate and precipitate out of solution (provided that low temperature conditions are maintained, and the protein is not irreversibly denatured). There are several ways to increase the hydrophobicity of proteins and selectively precipitate proteins, such as carefully controlling the addition of ammonium sulphate to the sample and preparative chromatography.

Selective precipitation of proteins can generally be applied as a bulk method to recover most of the proteins from the crude material, as a selective method to fractionate a subset of proteins from a protein-rich solution, and as a specific method to recover a single desired protein from a purification step (Nehete, et al., 2013).

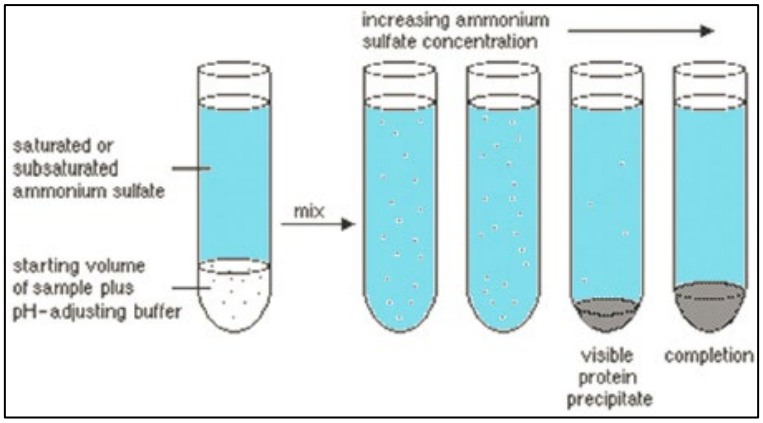


Figure 25: Lab-scale ammonium sulphate precipitation (Nehete, et al., 2013)

Ammonium precipitation is depicted in Figure 25. Proteins have different degrees of hydrophilicity thus it is possible to separate proteins in a mixture based on their relative hydrophilicity. For proteins with lower hydrophilicity, a lower concentration of ammonium sulphate will be necessary to aggregate and precipitate the protein. On the contrary, a higher saturation of ammonium sulphate will be necessary to precipitate highly hydrophilic proteins. Thus, by the gradual addition of different concentrations of ammonium sulphate (guided by salt fractionation tables), protein separation is possible.

Preparative chromatography typically involves using high-performance (or high pressure) liquid chromatography (HPLC) to separate different materials from a sample. This equipment can deliver high flowrates and usually includes a fraction-collection device to automatically collect extracts. Preparative chromatography is useful for the isolation of biological materials (like proteins) since great difficulty is experienced trying to isolate them by non-chromatographic methods. This technique enables the collection of different proteins based on peak fractions from a detector (Robards, et al., 2004).

2.4.4. Purifying protein extracts (removal of salts)

An issue experienced in the real-life application of organic coagulants in water treatment is the increase in organic matter in the water following coagulation and flocculation processes. In order to curb the occurrence of organic matter after treatment, it is proposed that the extracted coagulant proteins be further purified prior to water treatment (Camacho, et al., 2016). There are several methods that can be employed to accomplish this purification. Purification methods include dialysis, ultrafiltration, lyophilisation, ion-exchange, chemical precipitation and electrophoresis (Ndabigengesere, et al., 1995). Ion-exchange chromatography was used in a study by Ghebremichael, et al. (2005) to purify proteins. Although this is an effective method, the application of this method industrially may not be economically viable due to the high costs associated with chromatography. Ultrafiltration was investigated by Baptista, et al. (2015) as a means of protein purification by using differences in protein molar mass. When compared to chromatography, ultrafiltration is generally more low-cost, and simpler to use (Baptista, et al., 2015).

After separating proteins in the sample (via fractionation), the protein extract has a high concentration of ammonium sulphate salt. In order to remove the excessive salt, dialysis can be applied. Dialysis is a technique that enables the removal of minute particles from macromolecules in a solution, by a process of diffusion through a semi-permeable membrane (Thermofisher.com, 2020). By placing a solution containing the particles on one side of the membrane and a buffer solution (known as a dialysate) on the other, small particles pass through the pores in the membrane while macromolecules are retained. Particles move due to osmotic forces. This process continues until the small contaminants are reduced to acceptable levels. Therefore, by utilising the dialysis technique, the small ammonium sulphate particles can be removed into the buffer solution hence purifying the protein solution.

By replacing the buffer regularly and mixing the buffer solution, the process of dialysis can be expedited. Further, pre-treating dialysis tubing (by boiling with EDTA-$NaHCO_3$ solution) would prevent chemical contamination, which may greatly affect dialyzing extremely dilute protein solutions. The method of dialysis is favoured for its simplicity, however, this method has the disadvantage of being relatively slow and posing the inconvenience of requiring regular changes of the buffer solution (Scopes, 1994). Further, the salt removal process can lead to a loss in desired proteins (Lee, 2017).

It is difficult to isolate a particular desired protein for several reasons. Firstly, there is no generalised property that can be used to purify proteins, and several processing steps are conventionally required in the isolation process. Secondly, the desired protein is usually found in small quantities (in some cases picomolar concentrations), making analysis difficult due to instruments commonly being less sensitive. Thirdly, contamination issues occur frequently, where quantities of other proteins are found in the desired protein isolate. In some cases, there are more contaminants than target protein present in the isolate which causes complications in data analysis. Finally, since proteins are unstable, they are often degraded or denatured by unsuitable operating conditions (like temperature, pH, and ionic strength). Further, these proteins may aggregate before or after analysis if the operating conditions are not optimal (Lee, 2017). The general processing steps for plant-based coagulants are summarised in Figure 26.

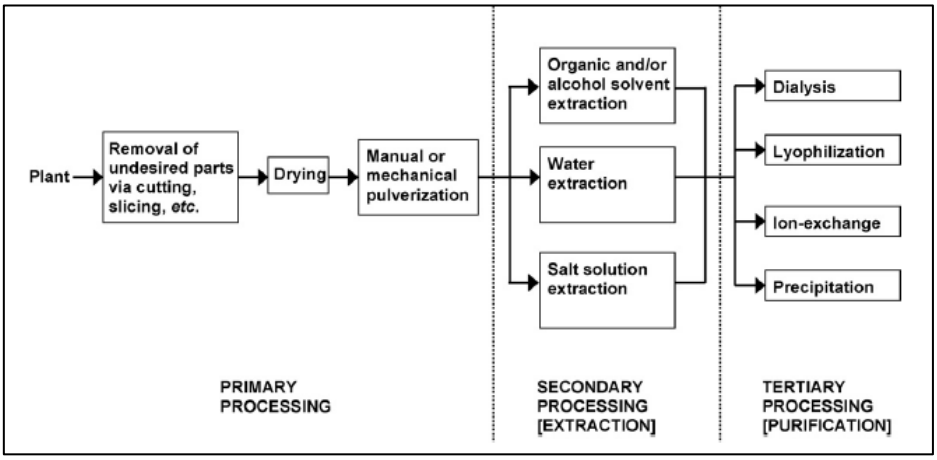


Figure 26: Processing steps for plant-based coagulants (Yin, 2010)

2.5. Protein loading (adsorption)

Immobilisation refers to the attachment of molecules to a particular surface which causes lowered mobility, or the complete loss of mobility. Proteins are associated with low stability and are easily denatured. Through immobilisation onto an inert surface, such as silica, proteins can be suitably protected against thermal denaturation, they can have increased protein activity and stability and they can be easily delivered to the desired place (Xu, et al., 2019). Several studies have shown that adsorption of seed proteins onto substrates can be a viable processing step in water treatment. Nordmark, et al. (2018) concluded that protein-modified sand would be stable upon reuse for water filtration making it an effective and sustainable water purification device. Therefore, heterogeneous coagulating agents were merited for their improved turbidity removal efficiency and retention of anti-microbial properties after treatment.

Proteins can be loaded onto a porous medium (such as silica) via three methods: immersion, impregnation and incipient wetness impregnation as shown in Figure 27. Immersion is a simplistic method that involves dissolving the protein molecules in a suitable solvent (such as water) and immersing the porous support (such as silica) into the solution. The protein becomes adsorbed onto the silica pore walls over a period of several hours. Thereafter, filtration, centrifugation and drying to remove the solvent takes place. This method does not require high temperature and is therefore very energy efficient and will not affect loading-sensitive molecules. For this method, relatively high concentrations of the loading solution may be necessary to achieve high loading degrees (which becomes problematic if the protein has poor solubility) (Lehto & Riikonen, 2014).

Impregnation involves the loading solution (containing the protein) firstly being mixed with the porous support and thereafter evaporating the solvent. The loading solution is drawn into the pores by capillary action and diffusion. This method is generally carried out in a rotavapor, fluidised bed or spray dryer. Impregnation offers the advantage of being extremely efficient however there are difficulties experienced in controlling the amount of protein loaded onto the surface of the porous material (Lehto & Riikonen, 2014).

The incipient wetness impregnation method refers to impregnation using a loading solution, with an approximately equal volume to the pore volume of the inert support. It sometimes involves loading molecules from a molten phase, but this is not generally applied as the molecules have to withstand temperatures higher than their melting point and since there is a high possibility of harmful reactions occurring at high temperatures. The incipient wetness impregnation method usually requires high loading solution concentrations and/or repeated impregnations to attain high loading degrees (Lehto & Riikonen, 2014).

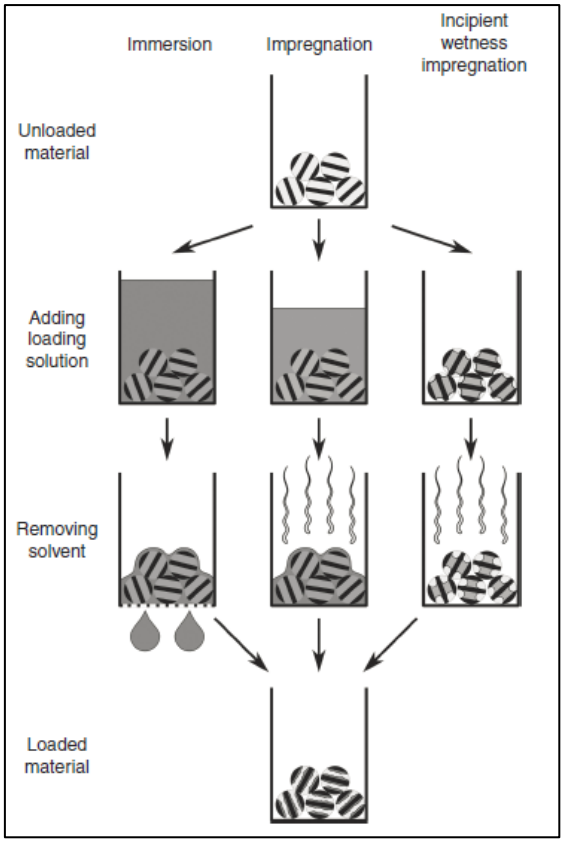


Figure 27: Methods used to carry out protein loading (Lehto & Riikonen, 2014)

In some cases, immobilisation of proteins may lead to the loss of protein activity (due to random orientation and structural deformation). Immobilisation is generally governed by three mechanisms, which are physical, covalent and bioaffinity immobilisation. Physical immobilisation is the adsorption of proteins onto surfaces by intermolecular forces (such as ionic bonds, hydrophobic interactions and polar interactions). Protein molecules are randomly oriented heterogeneously on the surface to reduce repulsion of the surface and existing adsorbed particles. The disadvantage of this mechanism is that proteins are generally weakly attached and can be removed by buffers. Further, there are issues with mass transport effects. Covalent immobilisation involves proteins covalently bonding to the support structure by functional groups of exposed amino acids. Chemical binding is usually random since it relies on residues present on proteins. Finally, bioaffinity immobilisation involves the loading of proteins in a gentle orientated and homogeneous manner that allows for the easy detachment of proteins, so that the surface can be reused (Rusmini, et al., 2007).

Protein adsorption commonly occurs as proteins are likely to adsorb when they contact solid interfaces. These proteins aggregate in solution and can be surface active even in low concentrations (Kwaambwa, et al., 2015). Globular proteins have both hydrophobic and hydrophilic characteristics which makes them strongly attracted to interfaces having either or both of these characteristics.

The adsorption process is highly dependent on the adsorbent (which is usually a porous solid medium such as silica), as the adsorbent's performance in equilibrium and kinetics determines the success of the process. It is imperative that the porous medium has good adsorptive capacity and kinetics to ensure that less adsorbent solid is required for a given throughput and that adsorbate molecules can travel to the particle interior at a good pace. In order to achieve this, adsorbents must satisfy the following criteria:

- The adsorbent must have an adequately high surface area or micropore volume (achieved by having a small pore size and reasonable porosity)
- The adsorbent must have a reasonably large pore network

Mesoporous silica has been industrially applied in separation, sensing, catalysis, and biotechnology (Niu, et al., 2009). Silica has been applied as an adsorbent in water treatment for its lowered toxicity, high protein loading capacity (due to large pore size), high surface area and large pore volume. It is important that the silica pore size be larger than the protein molecule to enable protein loading. A larger pore size tends to have enhanced protein loading. In order to expand pore size, polymers with longer carbon chains can be used as a template or organic swelling agents can be added (Xu, et al., 2019).

Loading of proteins onto the silica surface is generally due to the interaction between the surface and protein molecules through electrostatic interactions, hydrogen bonding, and pi-pi stacking (Xu, et al., 2019). The adsorption of proteins onto the surface of silica depends on the strength of the interaction between the protein and silica particles, the size of silica particles and the hydrophilic or hydrophobic nature of the silica particle surface.

Cationic proteins which have a positive net charge at a neutral pH are assumed to interact with the negatively charged silica substrate via electrostatic interactions. Two theories (stoichiometric displacement theory and counter-ion condensation theory) are generally used to describe the mechanism of electrostatic protein-substrate interactions without the influence of protein-salt interactions. The stoichiometric displacement theory states that physical interactions between the oppositely charged protein and substrate are necessary for adsorption and that adsorption takes place via ion-exchange (displacement of counter-ions) at the substrate surface. The counter-ion condensation theory states that proteins atmospherically interact with a counter-ion condensation layer that exists over the surface of an oppositely charged substrate and thereby never physically interact with the substrate.

Ideally, electrostatic attraction between oppositely charged protein and adsorbent surfaces favours adsorption. However, factors like the desolvation of charged groups, charge burying into a low dielectric medium and the osmotic pressure of counterions may oppose adsorption under these conditions.

Hydrophobic interactions between the protein and adsorbent surface play an important role in adsorption, where proteins with high hydrophobic residue content adsorb very strongly to hydrophobic surfaces (Hlady, et al., 1999). The stability of the protein depends on the hydrogen bonds among side chains, hydrophobic interactions among hydrophobic side chains, and charged residue interactions. It is possible that the adsorbent can compete for these interactions and unfold the protein structure leading to a minimisation of the free energy of the system (Hlady, et al., 1999).

Protein adsorption is a greatly dynamic phenomenon as proteins can alter its conformation and orientation during and after adsorption. The substrate surface properties affect protein adsorption, where proteins tend to be more influenced by hydrophobic or non-ionic surfaces rather than hydrophilic and polar surfaces (Lundström, 1985). How the protein behaves at a solid-water interface is influenced by factors such as the proteins' shape, size, surface charge, surface roughness (Hlady, et al., 1999), hydrophobicity, and thermodynamic stability. However, conformational stability and protein hydrophobicity (the strength of the hydrophobic interaction between the protein and solid substrate) majorly govern protein adsorption in a single-component system (Al-Malah, et al., 1995).

In most cases, more than one protein is present in solution therefore multi-protein adsorption has to be considered. Multi-protein adsorption is influenced not only by the interactions between proteins and the adsorbent but also by protein-protein interactions. These proteins often compete with each other for protein-surface interaction, which may lead to a transient dominance of one protein over the other proteins in solution. Since it is assumed that adsorbent surfaces have a finite surface capacity, these proteins compete for adsorption sites and proteins are often exchanged through adsorption-desorption processes (Hlady, et al., 1999). This is explained by the 'Vroman effect' where fast-diffusing proteins that interact to a lesser extent with the substrate are often replaced with slow-diffusing proteins that have a stronger interaction with the substrate. Cooperative adsorption that occurs in multi-protein systems involves complex adsorption kinetics making it difficult to describe protein adsorption at a molecular level through a generalised framework (Manzi, et al., 2019)

Protein adsorption can also occur when the protein and substrate are not oppositely charged. Adsorption at the wrong side of the isoelectric point may be due to differences in the pH of the bulk solution and near the adsorbent, which could promote interaction between the protein and adsorbent by the Donnan effect. The Donnan effect states that the formation of an electrical double layer around the charged adsorbent-liquid interface can either attract protons and exclude negative ions, and thereby reduce the local pH by up to 1 pH unit, or attract negative ions and exclude protons, and thereby increase the local pH by up to 1 pH unit. Therefore, the change in local pH around the adsorbent surface could change the net charge on the surface of the protein and promote electrostatic interactions and hence adsorption (Nfor, et al., 2010).

Adsorption of proteins at the wrong side of the isoelectric point can also be attributed to proteins having a patchy charge distribution (where some sides are positively charged, and others are negatively charged). Therefore, positively charged patches will be strongly attracted to negatively charged surfaces and vice versa. The number of charged groups in contact with the surface determine the binding strength of the protein to the surface. The degree of adsorption also depends on pH, where near the pI, the electrostatic repulsion among the protein molecules is minimised and the molecules can pack more tightly to the silica surface. The degree of adsorption onto the silica surface can be established by measuring protein depletion in solution after the addition of silica (by measuring protein concentration) or by measuring the change in the size of the silica particles (Meissner, et al., 2015).

Thus, the important parameters that influence the adsorption behaviour of proteins are:

- Protein properties: hydrophobicity, hydrophobic domain distribution of the protein surface, charge distribution on the protein surface, number and types of functional groups available for interaction with the surface groups, effective surface area available for adsorption, rigidity and stability
- Substrate properties: structural and chemical homogeneity, surface roughness, hydrophobicity, charge distribution on the substrate surface and effective surface area available for adsorption
- Solvent properties: dielectric constant, intermolecular forces, electrostatic forces, and environmental conditions (such as temperature, pH, ion concentration and protein concentration in solution)
- Solute properties: surfactant, excipients and stabilisers in solution tend to outcompete proteins for adsorption (Felsovalyi, 2012)

To gain a thorough understanding of protein adsorption, quantitative measures of characterisation and prediction, such as measuring adsorption isotherms, adsorption and desorption kinetics, the number of protein segments contacting the adsorbent surface, protein conformation upon adsorption and protein layer parameters(such as refractive index and layer thickness), can be employed (Hlady, et al., 1999). Adsorption studies of proteins are important as they provide vital information on how to utilise the protein material efficiently and ensure that there are reduced amounts of impurities and additives present (Kwaambwa, et al., 2010).

2.6. Literature summary

The lack of access to clean and safe drinking water in developing nations has prompted the need for a simple, low-cost and environmentally friendly method of front-end water treatment. A decentralised treatment system, involving the coagulation process, was deemed suitable for this purpose due to its effectiveness and simplicity. Inorganic coagulants are commonly applied in conventional coagulation processes however the high cost and health risks associated with chemical coagulants have justified research into natural coagulants. Several comprehensive studies surrounding the effectiveness of *Moringa Oleifera* as a natural coagulant exist in literature, however, limitations such as high dose requirements and low turbidity removal performance in low-to-medium turbidities have rationalised an investigation into other natural coagulants to overcome these limitations. In literature, *Glycine max, Cicer arietinum, Vigna unguiculata* and *Vigna mungo* were established as suitable candidates for testing as natural coagulants due to their high coagulation activity, non-toxicity, availability and biodegradability. Conventionally, active constituents from these plant species are aqueously extracted and directly applied as homogeneous coagulants to raw water. However, high dose requirements and poor floc settling characteristics associated with the use of homogeneous coagulants have prompted an investigation into heterogeneous coagulants. Heterogeneous coagulants prepared via immobilisation of proteins onto a silica substrate via wet impregnation were deemed effective in reducing turbidity, total hardness, chloride concentration and electrical conductivity and thus substantiate its use in this study. Protein recovery from plant material has to be optimised to ensure that sufficient active components were available for loading and hence coagulation. Aqueous extraction was established in literature as a simple, low-cost and effective means of recovering active components. However, to maximise protein extractability, salt extraction (via the salting-in process) was found to be a more effective alternative method. Salt precipitation (via the salting-out process) after salt extraction was also suggested in literature to optimise the loading process. The jar test is a simple yet effective means of evaluating the performance of coagulants and investigating optimum conditions for turbidity removal. Key factors such temperature, pH, mixing conditions, and coagulant dose need to be carefully controlled and optimised in the jar test to ensure effective coagulation and maximised turbidity removal.

CHAPTER 3: EXPERIMENTAL MATERIALS AND METHODOLOGY

An experimental design was developed based on the research presented in the literature review (chapter 2). Due to the extensive benefits associated with heterogeneous plant-derived coagulants such as *Moringa oleifera*, this experiment was designed to investigate the performance of heterogeneous coagulants derived from four other plant species (*Glycine max, Cicer arietinum, Vigna unguiculata and Vigna mungo).* The selection of each of these plant species is justified in section 3.1 of chapter 3. In order to develop effective heterogeneous plant-derived coagulants, extraction (aqueous extraction and salt extraction) and loading (wet impregnation) processes were employed as suggested by literature. An exploration of the extraction and loading processes are provided in sections 3.2 and section 3.3 respectively. Optimisation of these processes was performed using jar test apparatus to investigate the optimum salting-in and salting-out concentrations, and the optimum doses of heterogeneous coagulants produced via aqueous extraction and salt extraction. An outline of the experimental design for the determination of these optimum parameters is provided in section 3.4. Further, the materials and equipment employed in this study are listed in section 3.5 and experimental diagrams are provided in Appendix C. Finally, a detailed experimental procedure involved in the preparation of the plant species and synthetic turbid water, extraction and loading of active components and turbidity removal testing is provided in section 3.6.

3.1. Selection of plant species

Natural coagulants were preferred to chemical coagulants because they are eco-friendly and generally non-toxic. Further, Ndabigengesere & Narasiah (1998) found that aluminium sulphate produced 4 to 5 times more sludge than the natural coagulant *Moringa oleifera*, due to the formation of aluminium hydroxide as a precipitate when aluminium sulphate was used. The lower sludge volumes produced by natural coagulants (due to their sludge constituting mostly of agglomerated suspended particles and not additional precipitate) means that natural coagulants generally have lowered costs for handling and disposal of sludge. Since the by-products of natural coagulants are generally biodegradable organics, the sludge can be utilised as a fertiliser if there are no heavy metals in the treated water.

Natural coagulants generally do not consume alkalinity (as they are typically neutral to basic in nature), therefore pH adjustment costs and subsequent imbalances in the water chemistry are avoided. This was evidenced in a study by Ndabigengesere & Narasiah (1998), where the water alkalinity remained relatively constant with an increase in *Moringa oleifera* coagulant dose but decreased rapidly, in a separate experiment, when aluminium sulphate dose was increased.

There is a lack of extensive data regarding the toxicity of plant species (such as *J. curcas* and *Moringa oleifera*) which raises safety concerns, therefore, non-toxic or food-grade varieties of plants(legumes) were utilised in this study as a safer alternative (Hussain & Haydar, 2020).

Legume proteins were preferred for their nutritional value, low-cost, availability, and health benefits (Karaca, et al., 2011) Seed proteins were used as the protein content of legumes are generally higher than the rest of the plant.

Cicer arietinum was selected as candidate for the production of a heterogeneous coagulant due to its low cost, easily cultivation and availability. Further, the plant is biodegradable and safe for human consumption (Asrafuzzaman, et al., 2011). The seed of this plant has a high protein content of 25.3-28.9% (Chang, et al., 2012) and outperformed aluminium sulphate in water treatment; it showcased high turbidity removal of 98% in a study by Jaramillo & Epalza (2019). The high protein content and excellent coagulation activity thus made it a viable candidate for testing as a heterogeneous coagulant.

Cicer arietinum was also selected because it is drought-resistant and can grow in a wide range of soils, allowing for its easy application in many different regions. Currently, it is grown in over 57 countries which have varying environmental conditions (Merga, et al., 2019). Harvesting can be done approximately 100 days from sowing (Albert, 2020). In addition, harvesting can be easily carried out as trees grow up to a maximum of 1 meter in height. Although *Cicer arietinum* is one of the most widely grown crops, it is a major contributor to food security , therefore communities may be apprehensive about utilising the valuable food source for other applications (Zin, et al., 2018). However, its excellent properties for use as a coagulating agent (in relatively small doses) substantiate its applicability in water treatment.

Glycine max was selected as a candidate for turbidity removal due to its high protein content (40%) (Badole, et al., 2015), and subsequent high coagulation activity (98.8% turbidity removal (Mbogo, 2008)). In addition, *Glycine max* is considered to be one of the most low-cost sources of protein (Hussain & Haydar, 2020). The seeds are non-toxic and biodegradable therefore posing no harm to the environment or human health. This plant can be grown in a wide range of soils making it viable for application in different regions (Lim, 2012). *Glycine max* can be harvested between 45 to 65 days after sowing therefore raw plant materials can be quickly replenished (Albert, 2020). The harvesting itself can be easily done by hand since the plant only grows up to a height of 40-100cm (Badole, et al., 2015).

Glycine max production (particularly in South Africa) is anticipated to continue to grow therefore this plant is expected to become more readily available for its various uses. Currently, *Glycine max* production in South Africa has been majorly focussed on the manufacture of animal feed. *Glycine max* (raw and in the form of oil) has constituted a very small portion of South African household diets. The *Glycine max* seed is rarely consumed in rural communities in Southern Africa as there is limited knowledge of its food value in these areas (Dlamini, et al., 2014). Therefore, rural communities may be more inclined to consider the use of the crop for front-end water treatment rather than a food source.

Vigna unguiculata was chosen as a candidate for the development of a coagulant in turbidity removal due to its high protein content (2.5-31% (Lim, 2012)) and generally excellent performance as a coagulant in turbidity removal (99.2% (Mbogo, 2008)). Further, this legume is the third highest produced legume globally, making it easily accessible in most countries (Düzdemir, et al., 2009). In addition, *Vigna unguiculata* is non-toxic and does not require pH adjustment as it is not pH dependent (Liew, et al., 2004). *Vigna unguiculata* offers environmental benefits as it is capable of producing 2.5 times less sludge volume than aluminium sulphate. Further, the sludge produced by *Vigna unguiculata* may be spread on arable land so that the resources and nutrients can be recycled. In addition, the purified proteins showcase good antimicrobial properties making it useful for disinfection purposes (Blix, 2011).

Vigna unguiculata is favoured for its use in developing nations due to its drought-resistance (Jayalakshmi, et al., 2017). Further, the plant can tolerate highly acidic to highly basic soils (Lim, 2012). After pods dry (less than 100 days after sowing (E-TIC, 2020)), *Vigna unguiculata* can be easily harvested either using a V-blade on a tractor or by hoeing by hand (Farmer's weekly, 2016). The plant has a mean height of 117cm thereby allowing for easy harvesting of *Vigna unguiculata* (Onuh & Donald, 2009).

Vigna mungo was tested as a heterogeneous coagulant in this study because it has a high turbidity removal efficiency. In a study by Mbogo (2008), the plant achieved 100% turbidity removal thus outperforming aluminium sulphate and *Moringa oleifera.* Further, extractability and adsorption capacity were expected to be suitably high for this plant species due to its high protein content of 24.5-28.4% (Padhye, 1978).

Vigna mungo was also selected for its drought-resistance and versatility to be grown in a wide range of soils, which makes it suitable for use in developing nations (Lim, 2012). The legume is non-toxic to humans (it is widely consumed) and is biodegradable making it an environmentally friendly alternative to conventional coagulants. In addition, the legume is readily available as it has a high world production of approximately 3.2millions tons per year (Research and Markets, 2019). The crop also has a relatively short maturity time of 90 to 120 days, which allows for high production rates (Commodities control, 2020). Harvesting of the crop is relatively simple and can be done manually by hand as the crop grows up to 100cm high (Lim, 2012).

3.2. Extraction of active components

Experiments were performed to determine the optimum conditions for protein extraction from four plant species (*Glycine max, Cicer arietinum, Vigna unguiculata and Vigna mungo*). The unit operations used in the extraction of plant proteins play a vital role in extraction efficiency. In this study, comminution by grinding of the seeds was the first step. The purpose of grinding was to rupture the seed cells and release their constituents in order to improve protein extractability.

Intermittent grinding of each seed was carried out in a domestic coffee grinder to avoid excessive heat generation in the seed powder which could negatively impact the seed quality (Solanki, et al., 2005). The choice of dry grinding over wet grinding of the seeds is preferred in dry regions, such as the Sahara Desert and Africa, due to the low availability of water in these areas (Chelgani, et al., 2019). Further, due to the relatively low moisture content of the seeds under investigation, dry grinding of the seeds was carried out as suggested by Cater, et al. (1974).

The next stage in protein recovery from seed material is the extraction process. This step involved the dispersion of the seed powder in the extraction medium and the vigorously agitation of the solution. Vigorous agitation was employed to enhance the extraction of the active components into solution (Cater, et al., 1974). Following agitation, solid-liquid separation through sedimentation, centrifugation and filtration was necessary to enable efficient recovery of the active components in the supernatant, due to the relatively high solids content in the dispersion.

The extractability of proteins depends on the extraction medium, extraction pH, temperature, duration and environmental conditions (Hui, 1996). However, since the focus of this study was to solely determine the effect of the extraction medium on extractability, all other extraction conditions were kept constant. Temperature was maintained at room temperature since Hui (1996) suggests that temperature does not considerably affect protein extractability. Further, temperature was kept constant to avoid thermally altering the seed protein properties, which could have impacted its extractability, loading and coagulation activity. Similarly, a neutral pH was employed for this study and was not changed to avoid chemically altering the seed properties. It was imperative that the seed proteins were not damaged or inactivated through overly severe processing conditions (such as excessive heat) to prevent loss of valuable coagulating properties, and to avoid the possibility of the seed becoming toxic to humans (Cater, et al., 1974). Extraction time was kept constant at 45 minutes for all seed proteins. The extraction time was not extended as no considerable improvement in extraction generally occurs beyond this time (Hui, 1996) and froth formation during prolonged extraction (usually greater than one hour) may result in the denaturation and coagulation of the proteins (Sefa-Dedeh & Stanley, 1979).

This study employed two methods of protein extraction: aqueous extraction (using deionized water as the extraction medium) and salt extraction (using sodium chloride in deionized water as the extraction medium). These methods of extraction were selected due to the relatively high globulin and albumin content of the legumes under investigation, since these proteins are more readily extracted in salt solutions and water respectively.

3.3. Protein loading

Extracted proteins from each of the four plant species under investigation were loaded onto a silica substrate, via the wet impregnation method, to produce a heterogeneous coagulating agent. This involved the addition of a fixed mass of silica gel to solutions containing the extracted active components. Solutions were agitated to facilitate the adsorption process because mixing causes the concentration gradient of the protein in the bulk phase to disappear, which leads to the strong attachment of the protein from the solution onto the adsorbent surface (Sarkar & Chattoraj, 1993).The entire mass of silica substrate was added at once and the solution was agitated to encourage protein loading. The silica was not added gradually (at specific time intervals) as it was found by Lundström (1985) that protein adsorption is greater when the final concentration of proteins was added at once to the adsorbent rather than in a stepwise manner.

Protein adsorption is generally quantified by measuring the depletion of the solution after a period of equilibration with the adsorbent or by measuring the size increase (due to the formed protein layer) of the adsorbent. Measuring the size increase mitigates errors associated with measuring protein concentration however it requires the use of an appropriate isotherm equation (Meissner, et al., 2015).

Proteins were loaded onto a silica substrate to test the performance of the heterogeneous coagulant in turbidity removal. Therefore, a simple means of gauging and comparing protein adsorption at different ionic strengths was employed. This involved measuring the depletion of proteins in the protein-rich solution after loading, via ammonium precipitation. In the adsorption process, proteins become immobilised onto the silica substrate from the protein-rich solution (that was prepared from extraction). After filtration of the solution following wet impregnation, the proteins that were not adsorbed onto the substrate will be present in the filtrate. Thus, by precipitating the proteins in the filtrate, via ammonium precipitation, the degree of protein adsorption onto the substrate can be deduced. A lower quantity of precipitate formed indicates that a lower quantity of proteins is present in solution and therefore a higher quantity of proteins was adsorbed onto the silica substrate and vice versa.

Commercially available adsorbents such as activated carbon offer the benefit of being very effective however, they are expensive. Therefore, a low-cost alternative (silica gel) was considered for this application. A low-cost adsorbent is defined as such if it is abundant in nature, has low processing costs or can be obtained as a by-product or waste from another industrial application (Amin, et al., 2015). Since low-grade silica can be easily obtained from the natural environment, this study can be easily adapted for use in rural communities that do not have access to high grade chemicals.

Silica (SiO_2) is commonly utilised to study the interactions between proteins and inorganic surfaces. At a pH greater than 3, silanol groups are generally deprotonated as $Si\text{-}O^-$ which results in silica having a net negative charge (Mathe, et al., 2013). This is also substantiated by silica's isoelectric point being approximately 2 indicating that only neutral or negatively charged silica surfaces are available practically (Kwaambwa, et al., 2015). Silica was selected for its application as an adsorbent for its ability to be chemically modifiable to carry a particular chemical group, a protein or an immobilised ligand (Hlady, et al., 1999). In addition, silica is favoured for its low reactivity (Debnath, et al., 2011) and relative abundance in the Earth's crust. Nano-sized silica was not employed as they are potentially harmful to microflora in soil (Arumugam, et al., 2016).

A heterogeneous coagulant was investigated as it was found that Moringa proteins immobilised onto silica substrates offered several benefits in water treatment. The heterogeneous coagulant studied by Jerri, et al. (2012) was able to greatly remove turbidity and pathogens from water. In addition, the heterogeneous coagulant(which was found to have positive and negative charges on its surface) offered the advantage of dual functionality to remove both positively and negatively charged contaminant particles from a turbid solution (Nordmark, et al., 2018). Nordmark, et al. (2018) also indicated that adsorbed proteins and turbid particles can be desorbed from the silica surface and the adsorbent can be reused, which makes it a sustainable consideration for water treatment. Thus, this study seeks to assess protein immobilisation as a viable treatment step when applied to plant sources other than *Moringa oleifera*.

3.4. Experimental design

3.4.1. Experimental design for the optimum salting-in concentration for protein extraction

An investigation into the optimum conditions for extraction of the active components from the seeds of each biomass source was carried out according to the procedure described in section 3.6.5. Two iterations and an optimum repeat was performed in order to accurately establish the salt concentration at which protein extractability is maximised. The first iteration for all plant species involved the extraction of proteins in solutions with NaCl salt concentrations ranging from 0-0.9M as depicted in Table 4.

The range of salt concentrations used in the second iteration for each plant species was dependent on each plant's unique optimum salting-in concentration, as determined by the first iteration. The second iteration of salt extraction was carried out in a similar manner to iteration one, albeit over a narrower range of salt concentrations, that were in the vicinity of the optimum salting-in concentration, as determined in iteration 1.

Once the optimum salting-in concentration was accurately determined from the second iteration, an optimum repeat was performed. If the optimum salting-in concentration determined was denoted 'OSI', then the experimental points for the optimum repeat was as shown in Table 5. Aqueous extraction, in the absence of salt, was repeated three times as shown in Table 6.

It must be noted that the volume of distilled water and mass of biomass powder added to each sample was kept constant at 40ml and 2g respectively for all extraction samples. Ammonium precipitation of the protein-rich solution was used to establish the optimum salting-in concentration, which was the salt concentration that produced the highest mass of precipitate.

Table 4: Experimental points used in the first iteration for the determination of the optimum salting-in concentration for extraction of the active components from the seeds of each plant species

Sample number	Salt concentration (M)
1	0
2	0.1
3	0.3
4	0.5
5	0.7
6	0.9

Table 5: Experimental points used in the optimum repeat for the verification of the optimum salting-in concentration for extraction of the active components from the seeds of each plant species

Sample number	Salt concentration (M)
1	OSI[1]
2	OSI
3	OSI

Table 6: Experimental points used in the repetition run of the aqueous extraction of the active components from the seeds of each plant species

Sample number	Salt concentration (M)
1	0
2	0
3	0

[1] **OSI refers to the optimum salting-in concentration**

3.4.2. Experimental design for the optimum salting-out concentration for protein loading

Salting-out was investigated as a means of improving the loading efficiency of salt-extracted proteins onto a silica substrate. The optimum salting-out concentration was determined by incrementally increasing the salt concentration of the protein-rich solution and selecting the salt concentration at which protein loading was maximised. Two iterations and an optimum repeat were carried out for each plant species, in order to accurately establish their unique optimum salting-out concentration.

As depicted in Table 7, the first iteration in the determination of the optimum salting-out concentration firstly involved the preparation of samples containing proteins that were aqueously extracted (sample 1) and salt-extracted at the optimum salting-in concentration (OSI) (samples 2-6). Thereafter, the salt concentrations of the salt-extracted protein-rich samples 3 to 6, were incrementally increased beyond the optimum salting-in concentration (OSI) for each plant species. If the increment at which the NaCl concentration was incrementally increased beyond the optimum salting-in concentration (OSI) is denoted 'x', then the experimental points for the first iteration are as depicted in Table 7.

A second iteration was carried out over a narrower range of salt concentrations in the vicinity of the optimum salting-out concentration, as determined in the first iteration. An optimum repeat at the optimum salting-out concentration (denoted 'OSO') was carried out as shown in Table 8. Aqueous extraction and loading, in the absence of salt, was also repeated three times to verify the results, as shown in Table 9.

The adsorbent mass added to each sample, for loading, was established using a fixed mass of silica to solution volume ratio of 1.2g/9ml. Once proteins were loaded onto the silica substrate, via wet impregnation, the solution was filtered to obtain the heterogeneous coagulant product in the retentate. Ammonium precipitation of the filtrate solution from this process was used to establish the optimum salting-out concentration, which is the salt concentration that produces the lowest mass of precipitate in the filtrate, since a lower mass in the filtrate indicates the presence of more proteins in the retentate (heterogeneous coagulant product).

Table 7: Experimental points used in the first iteration for the determination of the optimum salting-out concentration for loading of the extracted active components onto a silica substrate

Sample number	Salt concentration at which coagulant was extracted M)	Salt concentration at which coagulant was loaded (M)
1	0	0
2	OSI	OSI
3	OSI	OSI + x[2]
4	OSI	OSI + 2x
5	OSI	OSI + 3x
6	OSI	OSI + 4x

Table 8: Experimental points used in the optimum repeat for the verification of the optimum salting-out concentration for loading of the extracted active components onto a silica substrate

Sample number	Salt concentration at which coagulant was extracted (M)	Salt concentration at which coagulant was loaded (M)
1	OSI	OSO[3]
2	OSI	OSO
3	OSI	OSO

Table 9: Experimental points used in the repetition run of the aqueous extraction and loading of the active components onto a silica substrate

Sample number	Salt concentration at which coagulant was extracted (M)	Salt concentration at which coagulant was loaded (M)
1	0	0
2	0	0
3	0	0

3.4.3. Experimental design for the optimum heterogeneous coagulant dose for turbidity removal

The optimum dose of heterogeneous coagulant (salt-extracted and loaded under optimum conditions) to be added to a synthetic turbid water sample was investigated. Two iterations and an optimum repeat was performed to determine the optimum dose at which turbidity removal was maximised.

[2] x refers to the increment at which NaCl concentration was increased beyond the optimum salting-in concentration

[3] OSO refers to the optimum salting-out concentration

The first iteration involved using visual experimental observation to establish the dose range at which the salt-extracted heterogeneous coagulant induces floc formation and does not increase the turbidity of the sample. A wide range of doses (0.1g/l to 100g/l) was considered, however the number of samples (denoted 'n') tested in this iteration was based on viewer experimental observations, which depended on the individual performance of each coagulant in turbidity removal. The experimental points used in the first iteration are shown in Table 10.

The range at which the optimum salt-extracted heterogeneous coagulant dose occurs, was determined in the first iteration and more accurately tested in the second iteration, over smaller dose increments. Finally, an optimum repeat at the optimum salt-extracted heterogeneous coagulant dose (denoted 'OSD') was carried out as shown in Table 11. An investigation into the optimum dose of aqueously extracted and loaded heterogeneous coagulant was similarly conducted as shown in Tables 12 and 13. The optimum aqueously extracted and loaded dose was denoted 'OAD'.

Turbidity removal testing was conducted using the jar test method and the initial turbidity of the synthetically prepared turbid samples (prior to coagulation) was fixed at 200 NTU. The lowest dose at which turbidity removal was maximised was deemed as the optimum heterogeneous coagulant dose.

In order to compare the coagulant results to that of natural settling, three samples (containing no coagulant) at an initial turbidity of 200 NTU was tested for turbidity removal performance over a period of time. The experimental points for the natural settling test are shown in Table 14.

Table 10: Experimental points used in the first iteration for the determination of the optimum salt-extracted and loaded heterogeneous coagulant dose of each plant species for turbidity removal

Sample number	Initial turbidity (NTU)	Salt concentration at which coagulant was extracted (M)	Salt concentration at which coagulant was loaded (M)	Coagulant dose (g/l)
1	200	OSI	OSO	0.1
2	200	OSI	OSO	0.2
⋮	⋮	⋮	⋮	⋮
n^4-1	200	OSI	OSO	99.9
n	200	OSI	OSO	100

[4] **n refers to the number of samples**

Table 11: Experimental points used in the optimum repeat for the verification of the optimum salt-extracted and loaded heterogeneous coagulant dose of each plant species for turbidity removal

Sample number	Initial turbidity (NTU)	Salt concentration at which coagulant was extracted (M)	Salt concentration at which coagulant was loaded (M)	Coagulant dose (g/l)
1	200	OSI	OSO	OSD[5]
2	200	OSI	OSO	OSD
3	200	OSI	OSO	OSD

Table 12: Experimental points used in the first iteration for the determination of the optimum aqueously extracted and loaded heterogeneous coagulant dose of each plant species for turbidity removal

Sample number	Salt concentration at which coagulant was extracted (M)	Salt concentration at which coagulant was loaded (M)	Coagulant dose (g/l)
1	0	0	0.1
2	0	0	0.2
⋮	⋮	⋮	⋮
n-1	0	0	99.9
n	0	0	100

Table 13: Experimental points used in the optimum repeat for the verification of the optimum aqueously extracted and loaded heterogeneous coagulant dose of each plant species for turbidity removal

Sample number	Salt concentration at which coagulant was extracted (M)	Salt concentration at which coagulant was loaded (M)	Coagulant dose (g/l)
1	0	0	OAD[6]
2	0	0	OAD
3	0	0	OAD

[5] OSD refers to the optimum salt-extracted heterogenous coagulant dose
[6] OAD refers to the optimum aqueously extracted heterogenous coagulant dose

Table 14: Experimental points used in the repetition run of the turbidity removal performance due to natural settling test

Sample number	Initial turbidity (NTU)	Coagulant dose (g/l)
1	200	0
2	200	0
3	200	0

3.5. Materials and equipment

3.5.1. Materials

3.5.1.1. Plant specimens

- *Vigna mungo* seed (shown in Figure 49 in Appendix C.1)
- *Cicer arietinum* seed (shown in Figure 50 in Appendix C.1)
- *Glycine max* seed (shown in Figure 51 in Appendix C.1)
- *Vigna unguiculata* seed (shown in Figure 52 in Appendix C.1)

3.5.1.2. Chemicals

- Sodium chloride (NaCl) (AR grade) obtained from Shalom Laboratory Supplies
- Ammonium sulphate ($(NH_4)_2SO_4$) with a chloride impurity content of 0.0005%, lead impurity of 0.0005%, sodium content of 0.002% and nitrate content of 0.001% acquired from Radchem (Pty) Ltd
- Silica gel (SiO_2) with 35-60 mesh particle size (high-purity grade – Davisil Grade 646) purchased from Sigma-Aldrich
- Kaolin clay powder (heavy powder CP grade) ($Al_2Si_2O_5(OH)_4$) supplied by Sigma-Aldrich
- Distilled water at room temperature provided by the chemical engineering laboratory at the University of KwaZulu Natal

3.5.2. Equipment

- Glassware: 100ml, 250ml and 1000ml glass beakers, volumetric measuring cylinders, glass vials, and watch glasses

- Other laboratory equipment: 50ml plastic centrifuge tubes, spatula, 600 µm metal sieve, and syringes

- Buchner funnel, Erlenmeyer flask, Whatman filter grade 13HW (70mm) and Edwards E2M1.5 vacuum pump (maximum outlet gauge pressure of 0.5 bar): The vacuum filter enabled the separation of a mixture of solid and liquid phases

- Grinding device: Russell Hobbs domestic coffee grinder to finely grind plant components for protein extraction (shown in Figure 53 in Appendix C.2)

- Digital scale: Kern & Sohn type ABS 220-4 (maximum capacity of 220g and readability of ±0.1mg). The digital scale enabled the measurement of sample masses (shown in Figure 54 in Appendix C.2)

- Mixing devices: Heidolph RZR 2014 mechanical overhead stirrer (speed range of 40rpm to 2000rpm), Labcon shaking incubator oven (speed range of 45rpm to 320rpm) (shown in Figure 55 in Appendix C.2) and Lasec reciprocating shaker (speed range of 25rpm to 250rpm) (shown in Figure 56 in Appendix C.2) to agitate mixtures

- Centrifuge device: Frontier multi centrifuge 5000 series (speed range of 200rpm-6000rpm) to separate solutions into a supernatant and solid residue phase (shown in Figure 57 in Appendix C.2)

- Turbidimeter: Lovibond infrared turbidimeter TB210 IR (turbidity measurement range of 0.01 to 1100 NTU) to facilitate turbidity analysis (shown in Figure 58 in Appendix C.2)

- Oven: Binder drying chamber to facilitate drying of samples to remove water

3.6. Experimental procedure

3.6.1. Start-up procedure

- ✓ All glassware was thoroughly cleaned to prevent contamination of samples.
- ✓ All containers were checked for leaks to avoid a loss of sample.
- ✓ Equipment was also inspected for damage to ensure fully functional operation during experimentation.

3.6.2. Preparation of the plant specimens for extraction

For the preparation of the plant specimens, the seed pods were left to naturally mature and dry. The seeds were carefully removed from the husks and sundried. Thereafter, good quality seeds with no signs of discoloration, softening or desiccation were selected. The chosen seed kernels were ground to a fine powder using a Russell Hobbs coffee grinder. Thereafter the ground seeds were sieved using a 600 µm metal sieve. The particles were kept at an approximate size off 600 μm to ensure solubility of the active proteins with the seed (Asrafuzzaman, et al., 2011). It is important to note that fresh samples of coagulant solutions were prepared before experimentation to prevent changes in pH, viscosity and coagulation activity due to microbial activity during storage (Ndabigengesere & Narasiah, 1998).

3.6.3. Preparation of the stock solution of synthetic turbid water

Kaolin clay powder was utilised to prepare synthetic turbid water samples. According to the preparation method used by Choudhary & Neogi (2017), 10g of kaolin clay powder was added to 1 litre of water and the suspension was stirred using the Heidolph mechanical overhead stirrer at 200rpm for 30 minutes. The suspension was thereafter allowed to settle for 24 hours to enable complete hydration of the kaolin particles. This suspension was used as a stock solution for the preparation of turbid water samples for turbidity testing.

3.6.4. Preparation of synthetic turbid water samples

In order to determine the dose of kaolin stock solution (with a concentration of 10g/l) to add to a fixed volume of distilled water to achieve the desired turbidity, turbidity testing was conducted at various stock solution doses. This involved the addition of varying volumes (0-20ml) of the supernatant of the stock solution to 200ml of distilled water. The prepared sample was then mixed using the Heidolph mechanical overhead stirrer and the turbidity was measured (using the Lovibond infrared turbidimeter) three times. The turbidity measurements were averaged and the relationship between stock solution dose and turbidity was established by linear regression.

The regressed linear equation was then used to determine the volume of stock solution that needs to be added to 200ml of distilled water to achieve the desired turbidity level of 200 NTU. Samples at the desired turbidity level were prepared freshly before jar tests, by adding the required dose of stock solution to 200ml distilled water, agitating and allowing 30 minutes for sedimentation.

3.6.5. Extraction of active components from seeds

Two methods of extraction were employed to recover the active components from the comminuted plant constituents. This included aqueous extraction (using distilled water as the extraction medium) and salt extraction (using sodium chloride solutions as the extraction medium). The general processing steps for each method were similar, with the exception of salt addition to distilled water for salt extraction. To establish the optimum salt concentration for extraction, the optimum salting-in concentration had to be determined for each plant species.

3.6.5.1. Determination of the optimum salting-in concentration for protein extraction

The first iteration of extraction involved the preparation of six centrifuge tubes that contained 40ml solutions each that had different salt concentrations (0M, 0.1M, 0.3M, 0.5M, 0.7M and 0.9M). The first centrifuge tube (which contained 0M NaCl) was used to facilitate aqueous extraction, while centrifuge tubes 2 to 6(with salt concentrations of 0.1M to 0.9M) were used to enable salt extraction. In order to achieve molarities of 0.1 M, 0.3 M, 0.5 M, 0.7 M and 0.9 M, the required mass of salt was computed and added to 40ml of distilled water. Centrifuge tubes containing the saline solutions were thereafter agitated in the Labcon shaking incubator oven until salts were completed dissolved in the water.

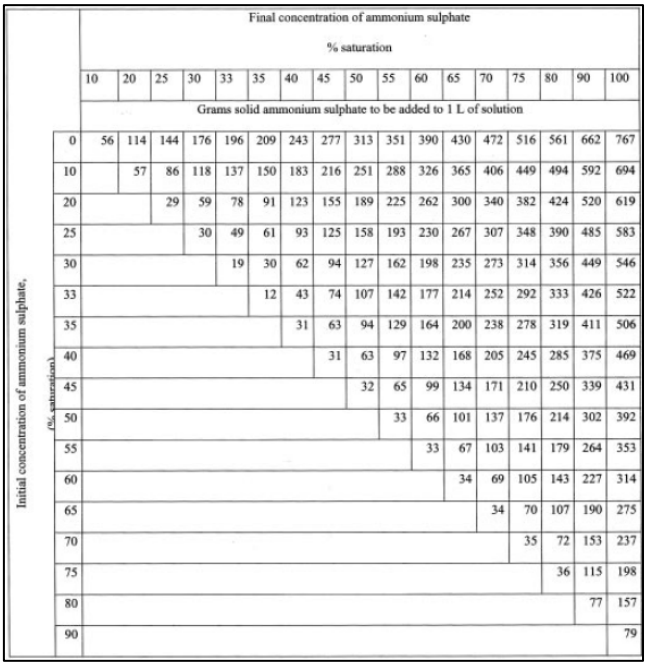
Approximately 2g of comminuted seed powder was added to each of the 6 centrifuge tubes and were vigorously agitated in the Labcon shaking incubator oven at a speed of 320rpm for 45 minutes. The centrifuge tubes were then left for 30 minutes to enable the sedimentation process. Centrifugation, using the Frontier multi centrifuge, was thereafter carried out for 45 minutes at a speed of 3500rpm. After which, the contents of each tube were filtered under suction using 13HW (70mm) Whatman grade filter paper. The filtrate was taken to be the crude solution of coagulant proteins.

3.6.5.2. Quantification of the amount of active components extracted

The ammonium sulphate($(NH_4)_2SO_4$) precipitation method was used to determine the amount of active components extracted aqueously and extracted using salt solutions. Using Figure 28, the required amount of ammonium sulphate to be added to 1 litre of water, to reach the desired levels of saturation, was determined and was scaled to the volume of filtrate produced after extraction.

A specific mass of ammonium sulphate (guided by Figure 28) was added to each of the six centrifuge tubes in order to saturate the solution from 0 to 20%. Thereafter, the solutions were mechanically agitated in the Labcon shaking incubator oven at 320 rpm for 15 minutes. This process was repeated whereby the ammonium sulphate saturation level was increased in 20% increments up to 100%, with intermediate mechanical stirring. Note that none of the supernatant solutions were extracted at each increment since protein fractionation was not the objective of this study.

Once complete ammonium sulphate saturation was attained, each solution was left undisturbed for 24 hours to aid precipitation. Thereafter, vacuum filtration using 13HW (70mm) Whatman grade filter paper was carried out. The solid retentate contained the protein of interest and was dried in the Binder drying chamber at 40˚C for 20 minutes. Thereafter, the mass of precipitate was determined using the KERN & Sohn digital scale. The mass of precipitate provided an indication of the optimum salting-in concentration for protein extraction as a high mass of precipitate indicated high extractability.



Initial concentration of ammonium sulphate, (% saturation)	Final concentration of ammonium sulphate, % saturation: 10	20	25	30	33	35	40	45	50	55	60	65	70	75	80	90	100
	Grams solid ammonium sulphate to be added to 1 L of solution																
0	56	114	144	176	196	209	243	277	313	351	390	430	472	516	561	662	767
10		57	86	118	137	150	183	216	251	288	326	365	406	449	494	592	694
20			29	59	78	91	123	155	189	225	262	300	340	382	424	520	619
25				30	49	61	93	125	158	193	230	267	307	348	390	485	583
30					19	30	62	94	127	162	198	235	273	314	356	449	546
33						12	43	74	107	142	177	214	252	292	333	426	522
35							31	63	94	129	164	200	238	278	319	411	506
40								31	63	97	132	168	205	245	285	375	469
45									32	65	99	134	171	210	250	339	431
50										33	66	101	137	176	214	302	392
55											33	67	103	141	179	264	353
60												34	69	105	143	227	314
65													34	70	107	190	275
70														35	72	153	237
75															36	115	198
80																77	157
90																	79

Figure 28: Nomograph used to determine the amount of ammonium sulphate required to attain specific percentages of saturation (Spardaro, et al., 2003)

The first iteration of extraction served to provide an estimate of the optimum salting-in concentration. A second iteration of salt extraction over a narrower range of salt concentrations was carried out to improve the accurately of the estimation of the optimum salting-in concentration for protein extraction. Once a more accurate estimate was determined, extraction at the optimum salting-in concentration was repeated three times to investigate the reproducibility of the results. Aqueous extraction was also repeated three additional times to verify the extraction performance of each plant species in the absence of salt. Note that the same general processing steps for extraction were used in iteration one, two and the optimum repeat.

3.6.6. Immobilization of extracted proteins onto silica substrates

The coagulant proteins were loaded onto an inert support to enhance settling and turbidity removal efficiency. The salting-out process was performed to improve the efficiency of protein loading onto a substrate. In order to determine the optimum conditions for maximised loading, the optimum salting-out concentration for each plant species was experimentally determined.

3.6.6.1. Determination of the optimum salting-out concentration for protein loading

The first iteration for the determination of the optimum salting-out concentration involved the preparation of five centrifuge tubes containing protein-rich solutions (extracted at the optimum salting-in concentration) and one centrifuge tube containing aqueously extracted active components. The extraction steps were similar to that presented in section 3.6.5.1 of the experimental methodology.

Following extraction, the salt concentration was increased above the optimum salting-in concentration in four of the salt-extracted protein-rich solutions. Thus, the first centrifuge tube contained 0M NaCl, the second centrifuge tube contained proteins extracted at the optimum salting-in concentration(the salt concentration was not increased further), and centrifuge tubes 3, 4, 5 and 6 had salt concentrations at the optimum salting-in concentration + x, optimum salting-in concentration + 2x, optimum salting-in concentration + 3x, optimum salting-in concentration + 4x respectively, where x was the increment at which the NaCl concentration was increased. The mass of NaCl to add to achieve the desired salt concentrations were determined using the solution volume and molecular mass of sodium chloride. After NaCl addition, each mixture was agitated in the Labcon shaking incubator oven until the salts were dissolved in the water.

Wet impregnation loading was thereafter carried out. This involved firstly adding a specific mass of silica gel to each centrifuge tube. The mass of silica gel to be added to the solution was determined by scaling the amount of silica added per protein-rich solution, used in Jerri, et al., (2012), which was 1.2g of silica per 9ml of solution, to the volume of protein-rich solution present after extraction. Following the addition of a specific mass of silica gel to each sample, the mixtures were agitated in the Labcon shaking incubator oven at 250 rpm for 60 minutes.

3.6.6.2. Quantification of remaining active components after loading

Once protein loading and agitation was complete, the solutions were filtered in a vacuum filter using Whatman filter paper grade 13HW (70mm). The filtrate from this process was used in ammonium precipitation to determine the amount of proteins that were not adsorbed onto the silica substrate. The ammonium precipitation method (as outlined in section 3.6.5.2 of the experimental methodology) was carried out.

The mass of precipitate formed via ammonium precipitation of the filtrate (remaining solution after loading) provided an indication of the optimum salting-out concentration, where a lower mass of precipitate formed from the filtrate indicated greater loading of proteins in the retentate.

A second iteration of salting-out was performed using a narrower range of salt concentrations to improve the accuracy of the optimum salting-out concentration estimation. Optimum repeats were carried out three times at the optimum salting-out concentration and in the absence of salt (aqueous system). Similar processing steps were used for iteration 2 and the optimum repeats.

3.6.7. Turbidity removal testing (jar tests)

Turbidity removal testing (jar testing) was used to determine the optimum dose of heterogeneous coagulant protein that would maximise turbidity removal performance. These tests were carried out at a fixed initial turbidity of 200 NTU. The initial turbidity of 200 NTU was selected to simulate natural water sources(such as rivers) that have a similar turbidity (Feria-Díaz, et al., 2016).

The salt-extracted heterogeneous coagulants (extracted at the optimum salting-in concentration and loaded at the optimum salting-out concentration) and aqueously extracted heterogeneous coagulants (extracted and loaded in distilled water) were produced using the processing steps outlined in section 3.6.6.1 of the experimental methodology. Following the agitation of the protein-rich solutions containing silica in the loading process, filtration was carried out in a vacuum filter using Whatman filter paper grade 13HW (70mm). The retentate from the filtration process was dried in the Binder drying chamber at 40˚C for 35 minutes. The dried solid granules from this process was the heterogeneous coagulant product that was utilised for testing.

3.6.7.1 Determination of the optimum heterogeneous coagulant dose for turbidity removal

Beakers containing 200ml of synthetic turbid water, with initial turbidities of 200 NTU, were prepared following the steps in section 3.6.4 of the experimental methodology. The heterogeneous coagulant product was added in varying doses to the synthetic turbid water. Each sample was rapidly agitated in the Lasec reciprocating shaker at 125rpm for 1 minute. The shaker speed was then reduced to 40rpm for 15 minutes to initiate the slow mixing stage. Finally, the sample was allowed to sediment for 60 minutes. In 10-minute intervals, during sedimentation, 10ml samples were withdrawn 3cm below the surface of the solution for turbidity analysis. Three turbidity readings of each sample were measured using the Lovibond infrared turbidimeter. The turbidity removal provided an indication of the optimum dose of coagulant. The lowest dose at which maximum turbidity removal was achieved was deemed the optimum dose.

Two iterations for the determination of the optimum coagulant dose were carried out for the aqueously extracted heterogeneous coagulant and for the salt-extracted heterogeneous coagulant. The first iteration for the determination of the optimum dose involved testing the turbidity removal of the aqueously extracted and salt-extracted coagulants over a wide range of doses (0.1-100g/l) and establishing, through visual experimental observation, the suitably low range of doses at which floc formation occurs and turbidity removal was high. The second iteration involved testing in a narrower range of doses to improve dose estimation accuracy. To showcase reproducibility, the jar tests were repeated three times at the optimum dose of coagulant (for both the aqueously extracted and salt-extracted heterogeneous coagulant). In order to compare the performance of the heterogeneous coagulant to natural settling, natural settling tests, involving synthetic turbid water samples that contained no coagulant, were carried out in a similar manner and repeated three times.

3.6.8. Shut down procedure

- ✓ All equipment was turned off, disconnected and stored.
- ✓ All waste materials and used chemicals were safely disposed according to standard waste disposal procedures.
- ✓ All glassware and other apparatus were thoroughly cleaned and stored.

CHAPTER 4: RESULTS

4.1. Extraction of active components

The ammonium precipitation method was utilised to quantify the degree of extractability of the active components from the seeds of the four plant species under investigation. The mass of precipitate formed after ammonium precipitation corresponds to the amount of protein extracted. For each plant species, three runs of aqueous extraction were carried out. Further, two iterations of salt extraction were performed to establish the optimum salting-in concentration for maximum extraction. In addition, the extraction process was repeated three times at the optimum salting-in concentration.

4.1.1. Aqueous and salt extraction of four plant species

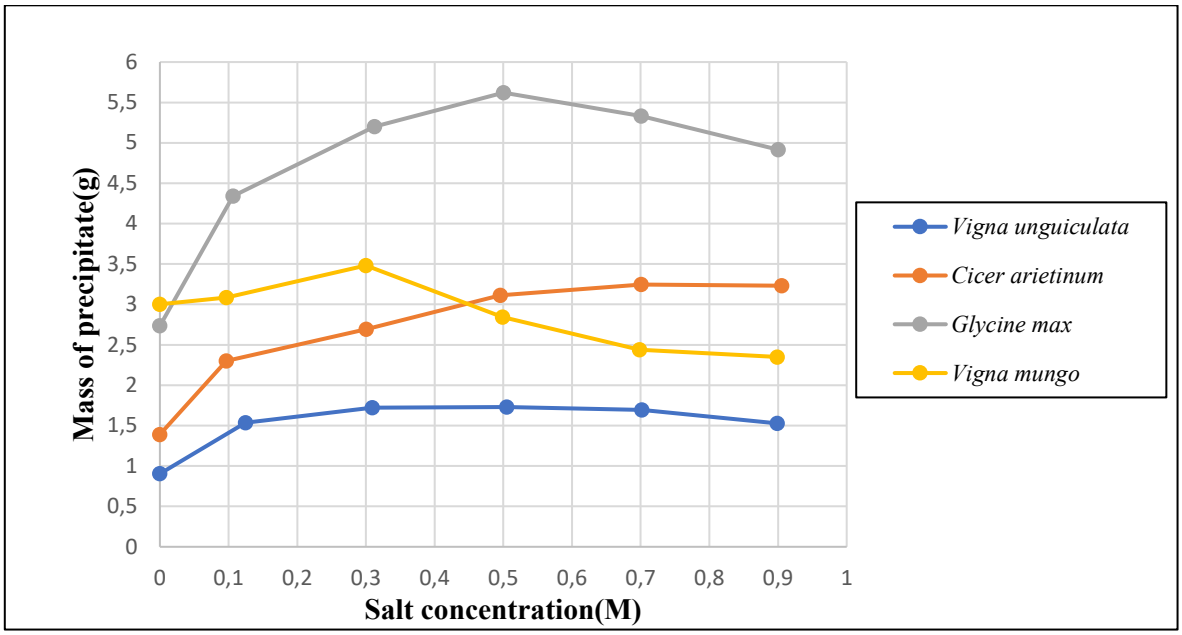


Figure 29: The mass of precipitate formed at varying salt concentrations (0-0.9M) following extraction of the active components from the seeds of four plant species

Figure 29 depicts the results of the first iteration which involved the salt and aqueous extraction of the active components from the seeds of the four plant species under investigation. The results showed that in general, *Glycine max* produced the greatest amount of precipitate (between 2.735g and 5.621g) for salt concentrations greater than 0M. It was also observed that *Vigna unguiculata* produced the least amount of precipitate (between 0.904g and 1.73g) at all salt concentrations. *Vigna mungo* provided a greater amount of precipitate than *Cicer arietinum* in the salt concentration range of 0M to approximately 0.45M, thereafter *Cicer arietinum* produced a greater amount of precipitate with increasing salt concentration. It was noted than *Vigna mungo* produced the greatest amount of precipitate compared to the other plant species, when the active components were aqueously extracted. In addition, for *Vigna mungo*, the mass of precipitate produced by extraction using saline solutions, that had concentrations of 0.5M-0.9M, was lower than that produced by aqueous extraction.

4.1.2. Salt extraction

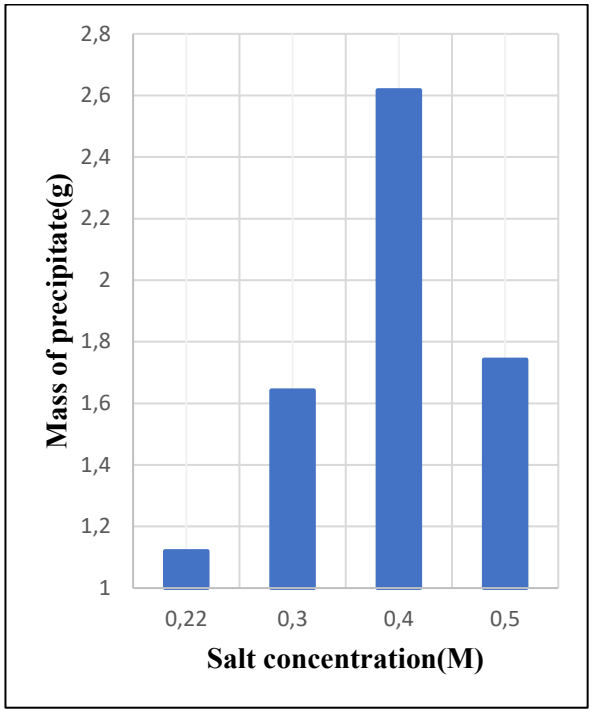


Figure 30: The mass of precipitate formed after extraction of *Vigna unguiculata* active components at salt concentrations ranging from 0.22M to 0.5M

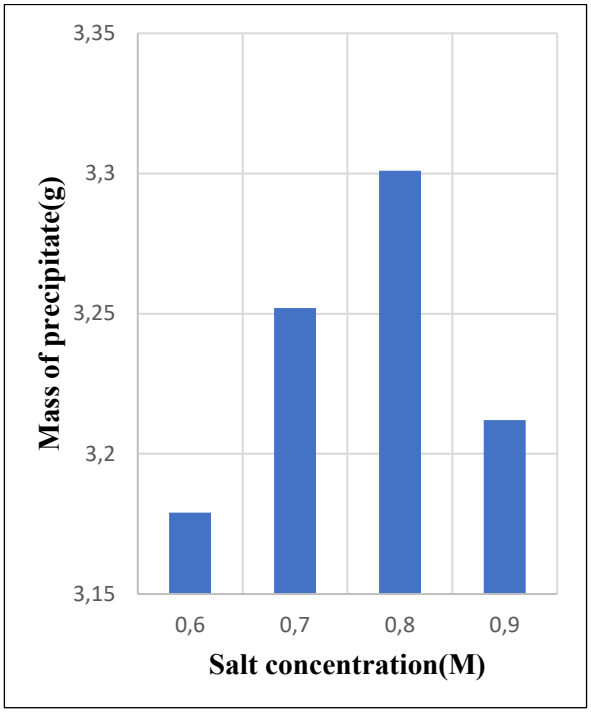


Figure 31: The mass of precipitate formed after extraction of *Cicer arietinum* active components at salt concentrations ranging from 0.6M to 0.9M

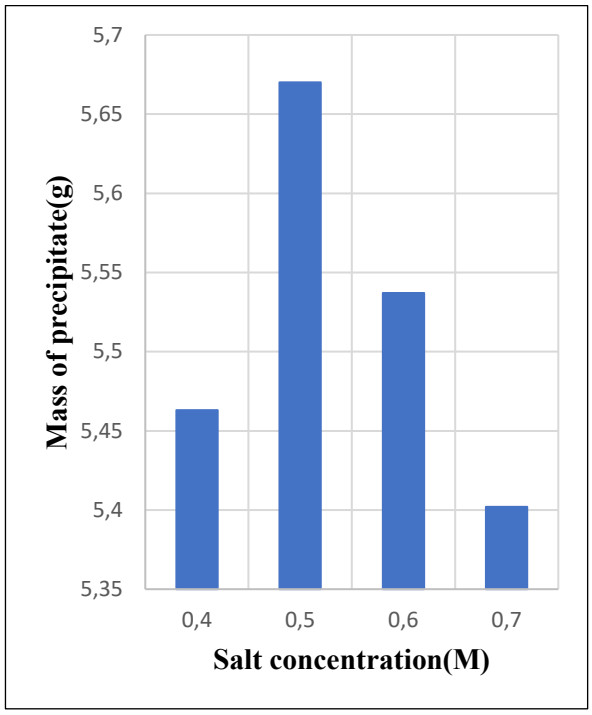


Figure 32: The mass of precipitate formed after extraction of *Glycine max* active components at salt concentrations ranging from 0.4M to 0.7M

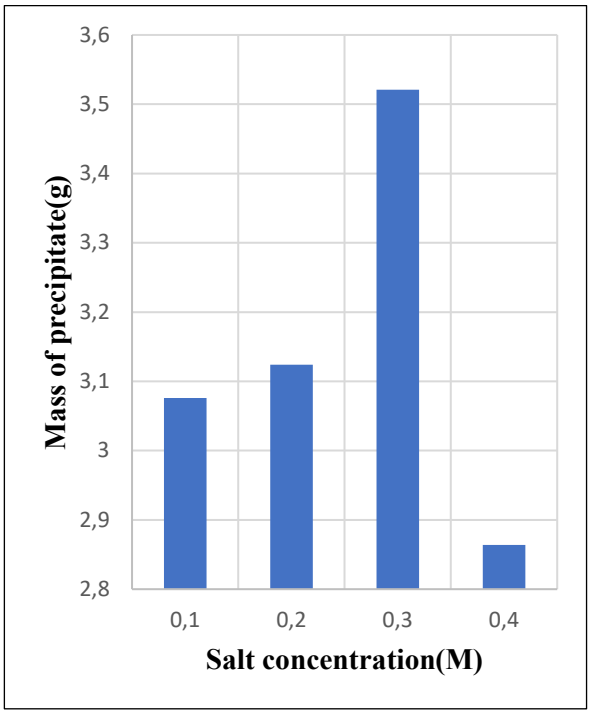


Figure 33: The mass of precipitate formed after extraction of *Vigna mungo* active components at salt concentrations ranging from 0.1M to 0.4M

Figures 30-33 show the results of the second iteration of salt-extraction for each plant species. This iteration was performed over a narrower salt concentration range to establish the optimum salting-in concentration. The results produced are similar to the findings in the first iteration (shown in Figure 29) which indicates good repeatability. The results for the salt extraction of the active components from *Vigna unguiculata* (as depicted in Figure 30) show that as the salt concentration increases, the mass of precipitate formed increases, up to a salt concentration of 0.4M. Thereafter, the mass of precipitate formed decreases with increasing salt concentration (from 0.4-0.9M) as shown in Figure 29. Similar results are shown for *Cicer arietinum, Glycine max* and *Vigna mungo* in Figures 31, 32, and 33 respectively, however, the graph for each plant species has a different turning point. It can be determined from Figures 31, 32 and 33 that the graph peaks (where the mass of precipitate formed is at a maximum) for *Cicer arietinum, Glycine max* and *Vigna mungo* are at salt concentrations of 0.8M, 0.5M and 0.3M respectively.

Table 15: Average mass of precipitate formed after salt extraction (at the optimum salting-in concentration) of the active components from four plant species

Plant species	Optimum salting-in concentration(M)	Average mass of precipitate formed(g) ±standard deviation
Vigna mungo	0.3	3.513±0.009
Glycine max	0.5	5.654±0.362
Vigna unguiculata	0.4	2.358±0.455
Cicer arietinum	0.8	3.124±0.201

Salt extraction at the optimum salting-in concentration was repeated three times and the average mass of precipitate ±the standard deviation of 3 samples is shown in Table 15. The results show that *Cicer arietinum* required the highest salt concentration (0.8M) to maximise its production of precipitate. However, a lower salt concentration of 0.5M was required to produce a greater mass of precipitate in the case of *Glycine max.* For *Vigna Mungo,* the lowest salt concentration of 0.3M was required to maximise precipitate formation. The standard deviations computed were relatively small indicating good repeatability.

4.1.3. Aqueous extraction

Three runs of aqueous extraction were carried out and the average mass of precipitate formed by ammonium precipitation for each plant species is depicted in Table 16. The results show that the extraction of the active components from *Vigna mungo* seeds produced the largest mass of precipitate (3.103g), while *Vigna unguiculata* produced the least amount of precipitate (1.124g) compared to the other plants species under investigation. The standard deviations were low indicating good repeatability.

Table 16: Average mass of precipitate formed after aqueous extraction of active components from the seeds of four plant species

Plant species	Average mass of precipitate formed(g) ±standard deviation
Vigna mungo	3.103±0.209
Glycine max	2.612±0.231
Vigna unguiculata	1.124±0.156
Cicer arietinum	1.342±0.037

The protein composition and sodium content of each plant species was obtained from literature and is summarised in Table 17. These seed properties were used to explain the extractability of each plant species.

Table 17: Protein composition and sodium content of the seeds of four plant species

Plant species	Protein content (%)	Globulin content (%)	Albumin content (%)	Sodium content (g/100g seed)
Vigna mungo	28.4 (Padhye, 1978)	81 (Padhye, 1978)	13 (Padhye, 1978)	38 (Nutrition Value, 2020)
Glycine max	40 (Badole, et al., 2015)	46.5 (Ciabotti, et al., 2016)	31.8 (Ciabotti, et al., 2016)	2 (Nutrition Value, 2020)
Vigna unguiculata	31 (Lim, 2012)	66.6 (Agustin, et al., 2020)	24.9 (Agustin, et al., 2020)	16 (Nutrition Value, 2020)
Cicer arietinum	28.9 (Chang, et al., 2012)	56 (Chang, et al., 2012)	12 (Chang, et al., 2012)	24 (Nutrition Value, 2020)

4.2. Loading of proteins onto a silica substrate

In order to quantify the degree of adsorption, ammonium precipitation was employed as a measure of the protein concentration in the depleted solution, following loading of the proteins derived from the four plant species studied. The mass of precipitate formed corresponds to the amount of protein not adsorbed onto the silica substrate, therefore a lower mass of precipitate indicates greater protein adsorption. Three runs of loading (and ammonium precipitation) of the aqueously extracted proteins were performed. In addition, two iterations of salting-out was carried out to establish the optimum salting-out concentration for maximised protein adsorption. Loading at the optimum salting-out concentration, and ammonium precipitation was repeated three times.

4.2.1. Aqueously extracted and salt-extracted heterogeneous coagulant

Figure 34 shows the results of the first iteration of protein adsorption measurements, for the four plant species under investigation. The first iteration involved carrying out loading in the absence of salt (aqueous loading) and at varying salting-out concentrations (above the optimum salting-in concentration). Figure 34 shows an increase in the amount of precipitate formed at salt concentrations greater than 0.4M, in the case of *Vigna unguiculata*. For *Cicer arietinum*, it can be viewed from Figure 34, that with increasing salt concentration (in the range of 0.8-0.9M), the mass of precipitate formed increases, and thereafter does not change significantly at salt concentrations higher than 0.9M. For *Glycine max*, the mass of precipitate decreases with increasing salt concentration (from 0.5-0.6M), minimises at a salt concentration of 0.6M, and decreases from 0.6-0.7M. The mass of precipitate formed does not increase significantly when the salt concentration was increased beyond 0.7M.

The results in Figure 34 show that for all plant species, the mass of precipitate formed after aqueously loading the proteins was higher than that formed when proteins were loaded using solutions that were at the optimum salting-out concentration for each plant species. It must be noted that the mass of precipitate formed after loading of the aqueously extracted *Vigna unguiculata* proteins was lower than that produced by loading in saline solutions with concentrations greater than 0.5M.

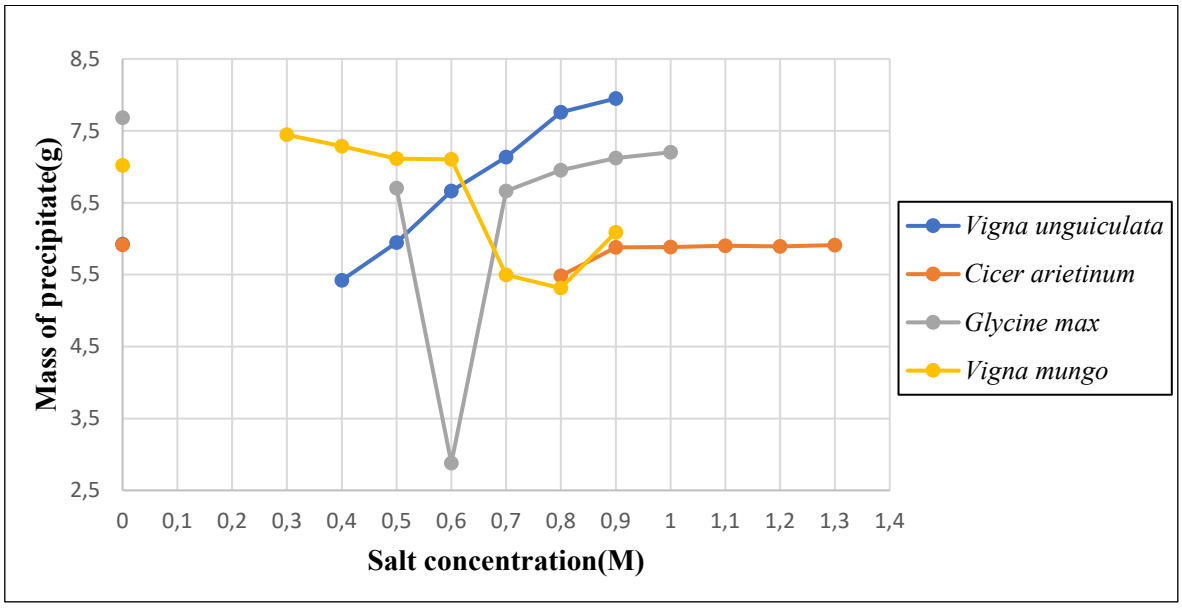


Figure 34: The mass of precipitate formed from the filtrate of the aqueously loaded and salt loaded (at varying salt concentrations beyond the optimum salting-in concentration) active components extracted from the seeds of four plant species

4.2.2. Salt-extracted heterogeneous coagulant

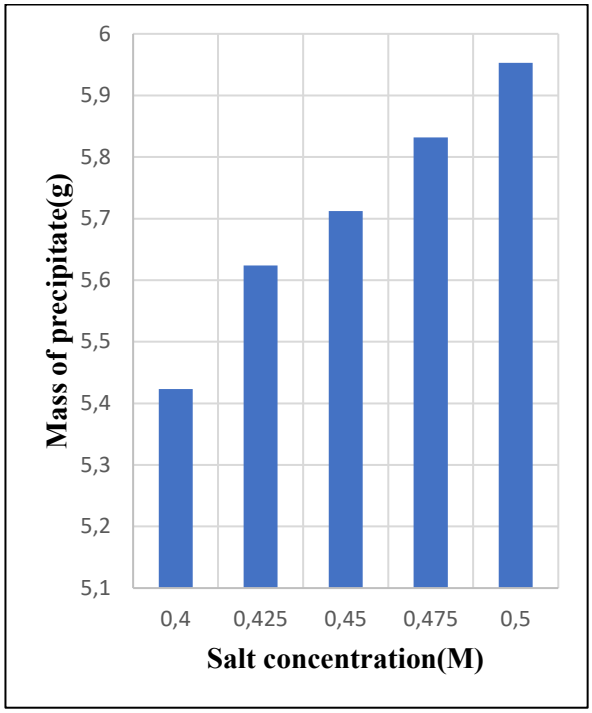


Figure 35: The mass of precipitate formed after loading of *Vigna unguiculata* active components at salt concentrations ranging from 0.4M to 0.5M

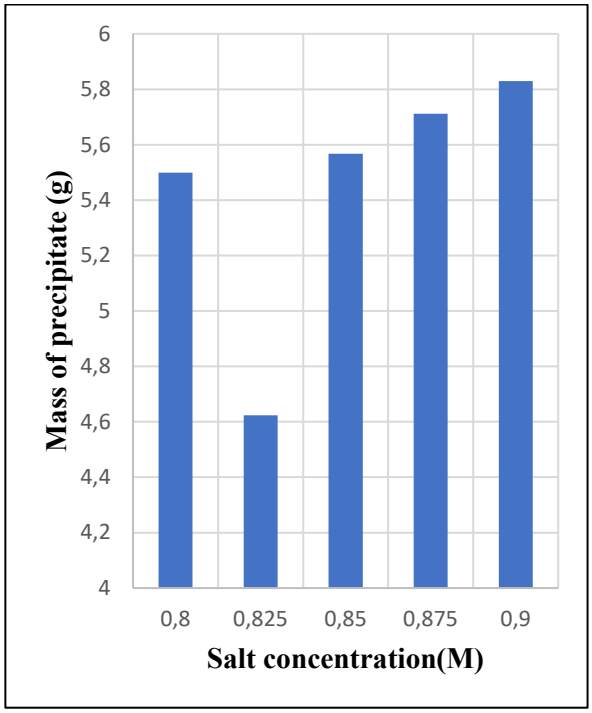


Figure 36: The mass of precipitate formed after loading of *Cicer arietinum* active components at salt concentrations ranging from 0.8M to 0.9M

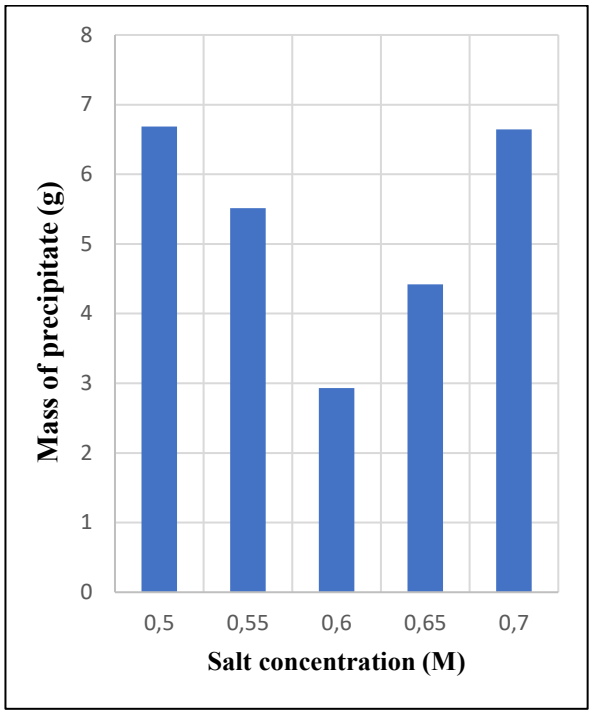


Figure 37: The mass of precipitate formed after loading of *Glycine max* active components at salt concentrations ranging from 0.5M to 0.7M

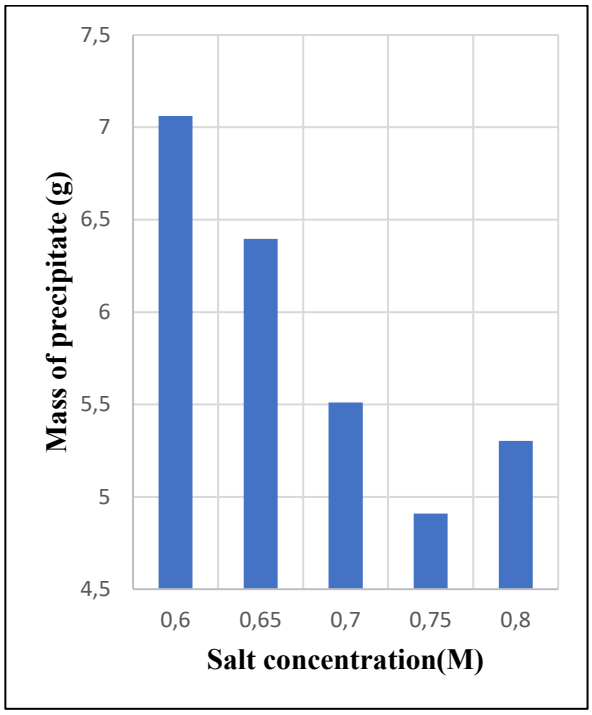


Figure 38: The mass of precipitate formed after loading of *Vigna mungo* active components at salt concentrations ranging from 0.5M to 0.8M

Figures 35-38 show the results of the second iteration of protein adsorption measurements (to determine the optimum salting-out concentration) for each plant species. This iteration was carried out over a narrower salt concentration range than iteration 1. These results showcase good repeatability as the adsorption results were comparable to that shown in Figure 34.

The results in Figure 35 show that for *Vigna unguiculata*, the mass of precipitate formed increases with increasing salt concentration past the optimum salting-out concentration (0.4M). The results for *Cicer arietinum*, *Glycine max* and *Vigna mungo* (shown in Figures 36,37 and 38 respectively) show that the mass of precipitate produced decreases with increasing salt concentration up to a specific concentration and thereafter increases. This specific concentration (where the graph minimises) was deemed as the optimum salting-out concentration and was determined as 0.825M, 0.6M and 0.75M for *Cicer arietinum*, *Glycine max* and *Vigna mungo* respectively.

Table 18: Average mass of precipitate formed from the filtrate after filtration of the salt loaded (at the optimum salting-out concentration) coagulants derived from the active components of four plant species

Plant species	Optimum salting-out concentration(M)	Average mass of precipitate formed(g) ±standard deviation
Vigna mungo	0.75	4.914±0.005
Glycine max	0.6	2.926±0.036
Vigna unguiculata	0.4	5.482±0.083
Cicer arietinum	0.825	4.715±0.076

Loading and ammonium precipitation at the optimum salting-out concentration was repeated three times for each plant species. The average mass of precipitate formed when proteins were loaded at the optimum salting-out concentration is shown in Table 18. Good repeatability was indicated by the low standard deviations. *Glycine max* produced the lowest average mass of precipitate (2.926g) at a lower optimum salting-out concentration than *Vigna mungo* and *Cicer arietinum*. The optimum salting-out concentration was the same as the optimum salting-in concentration in the case of *Vigna unguiculata* and this plant species produced the highest average mass of precipitate (5.482g) compared to the other plant species.

4.2.3. Aqueously extracted heterogeneous coagulant

Table 19: Average amount of precipitate formed from the filtrate of the aqueously loaded coagulants derived from the active components of four plant species

Plant species	Average mass of precipitate formed(g) ±standard deviation
Vigna mungo	7.126±0.076
Glycine max	6.925±0.576
Vigna unguiculata	5.931±0.007
Cicer arietinum	5.919±0.004

To investigate repeatability, loading of the aqueously extracted proteins and ammonium precipitation was carried out three times. The results shown in Table 19 indicate that *Vigna mungo* produced the highest average mass of precipitate (7.126g) while *Cicer arietinum* produced the lowest average mass of precipitate (5.919g) relative to the four plant species investigated. The low standard deviation indicated low variability in results upon repetition.

The isoelectric point and amino acid composition, of the plant species under investigation, were extracted from literature sources and summarised in Table 20 and 21. These protein properties were used to explain protein adsorption behaviour upon loading onto a silica substrate.

Table 20: Isoelectric point and net charge of seed proteins at a neutral pH

	Isoelectric point	Net charge at neutral pH
Plant species		
Vigna mungo	4-5 (Kamani, et al., 2019)	Negative
Glycine max	4-5 (Jaramillo, et al., 2011)	Negative
Vigna unguiculata	4.5 (Abdel-Shafi, et al., 2019)	Negative
Cicer arietinum	3.7 (Jaramillo, et al., 2011)	Negative
Adsorbent		
Silica gel	2 (Kwaambwa, et al., 2015)	Negative

Table 21: Amino acid composition of seed proteins

	Vigna mungo (Nwosu, et al., 2019)	*Glycine max* (Kovalenko, et al., 2006)	*Vigna unguiculata* (Chan & Phillips, 1994)	*Cicer arietinum* (Dhawan, et al., 1991)
Positively charged amino acid content (%)				
Lysine	6.7	2.7	7.1	6.9
Arginine	7.1	3.2	7.1	9.4
Histidine	2.2	1.6	2.6	2.3
Total	**15.9**	**7**	**16.8**	**18.6**
Negatively charged amino acid content (%)				
Aspartic acid	12.1	4.8	12.6	11.4
Glutamic acid	13.6	7.7	19.5	16.5
Total	**25.7**	**12.5**	**32.1**	**27.8**

4.3. Turbidity removal testing

Different doses (in the range of 0-20ml) of kaolin stock solution (with a concentration of 10g/l) were added to 200ml samples of distilled water and the turbidity was measured three times upon addition of each subsequent dose. This was carried out to determine the relationship between turbidity and stock solution dose so that the dose of stock solution to achieve a desired turbidity could be easily determined. Figure 39 showcases a strong positive linear relationship between average turbidity and the volume of stock solution added to 200ml of distilled water.

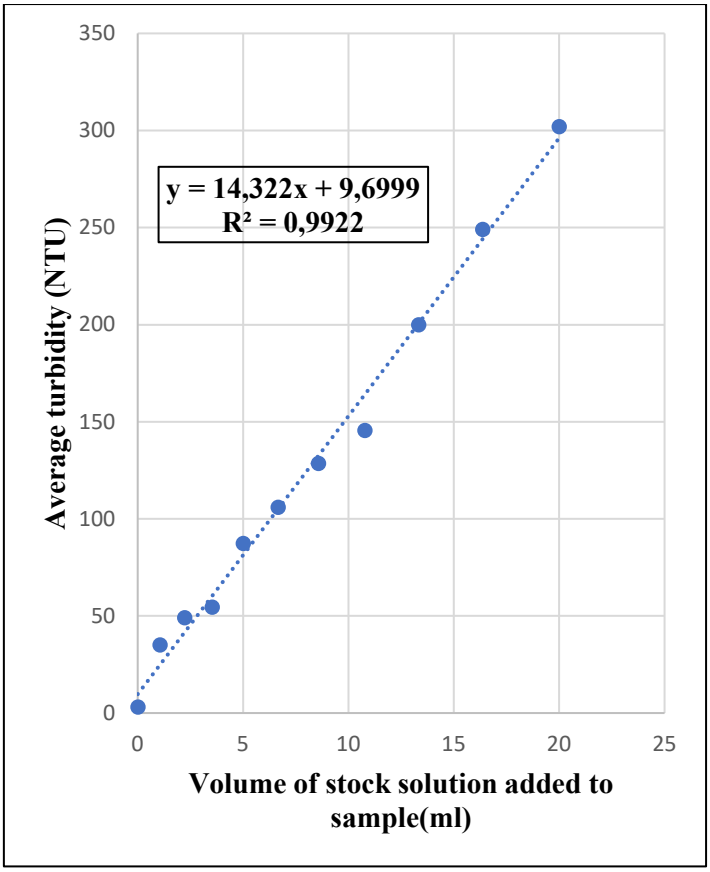


Figure 39: Relationship between average turbidity and volume of stock solutions added to a 200ml sample of distilled water

4.3.1. Salt-extracted heterogeneous coagulant

In order to assess the turbidity removal performance (and determine the optimum dose) of the salt-extracted heterogeneous coagulant developed, jar tests were carried out. The jar tests involved the addition of different doses of the coagulant into a 200ml sample of turbid water that had an initial turbidity of 200 ± 0.5 NTU. Thereafter, rapid and slow mixing was carried out followed by a 60-minute sedimentation period. In the sedimentation period, three turbidity measurements were carried out every 10 minutes. An initial dose of range of 0.1-100g/l was tested to determine the coagulant dose at which floc formation occurs. Figures 40 to 43 show the second iteration of jar tests. These Figures indicate the average turbidity removal percentages of the coagulants, at doses ranging from 2.5-25g/l. In general, turbidity removal increases with increasing dose and maximises at a specific optimum dose, thereafter the graph decreases with increasing dose. In Figures 40 and 43, it was found that at a dose of 5g/l, maximum turbidity removal percentages of 83% and 89.1% were achieved for *Vigna unguiculata* and *Vigna mungo* respectively. In Figures 41 and 42, *Cicer arietinum* and *Glycine max* achieved maximum turbidity removal percentages of 79.4% and 87% at a higher dose of 10g/l. The turbidity removal percentages for all plant-derived salt-extracted heterogeneous coagulants were substantially higher than that achieved by natural settling (which had a maximum turbidity removal percentage of 4.1% after 60 minutes).

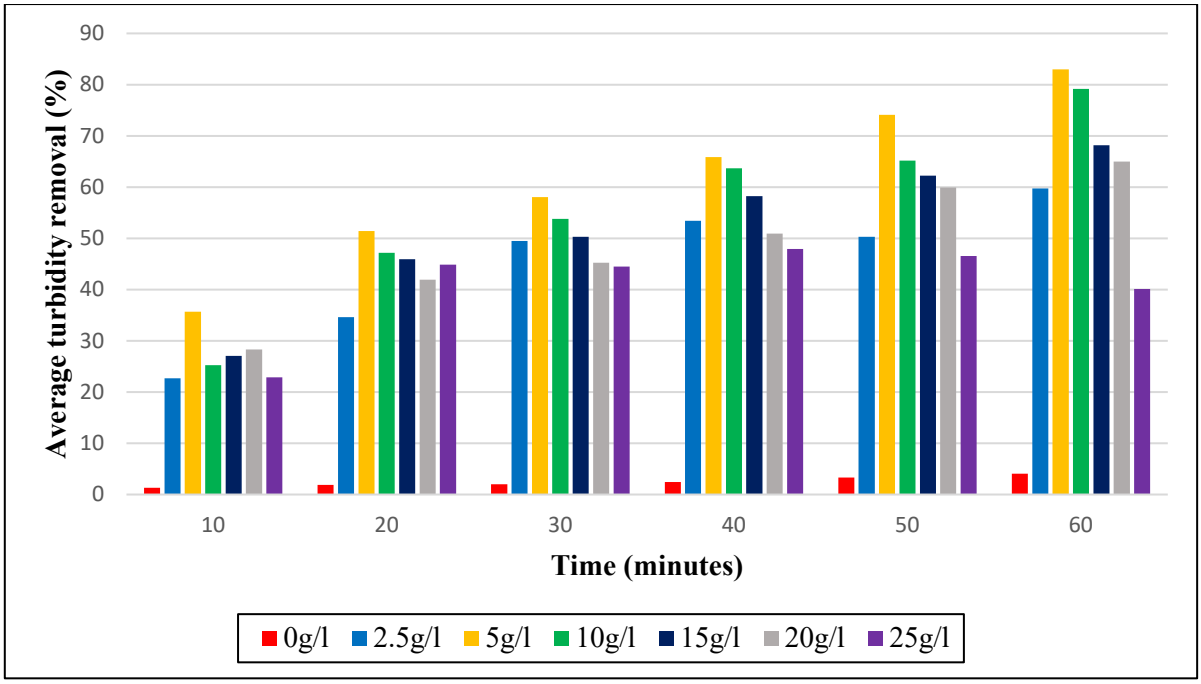


Figure 40: The effect of the dose of salt-extracted heterogeneous coagulant derived from *Vigna unguiculata* on turbidity removal (from an initial turbidity of 200 ± 0.5 NTU) over 60 minutes

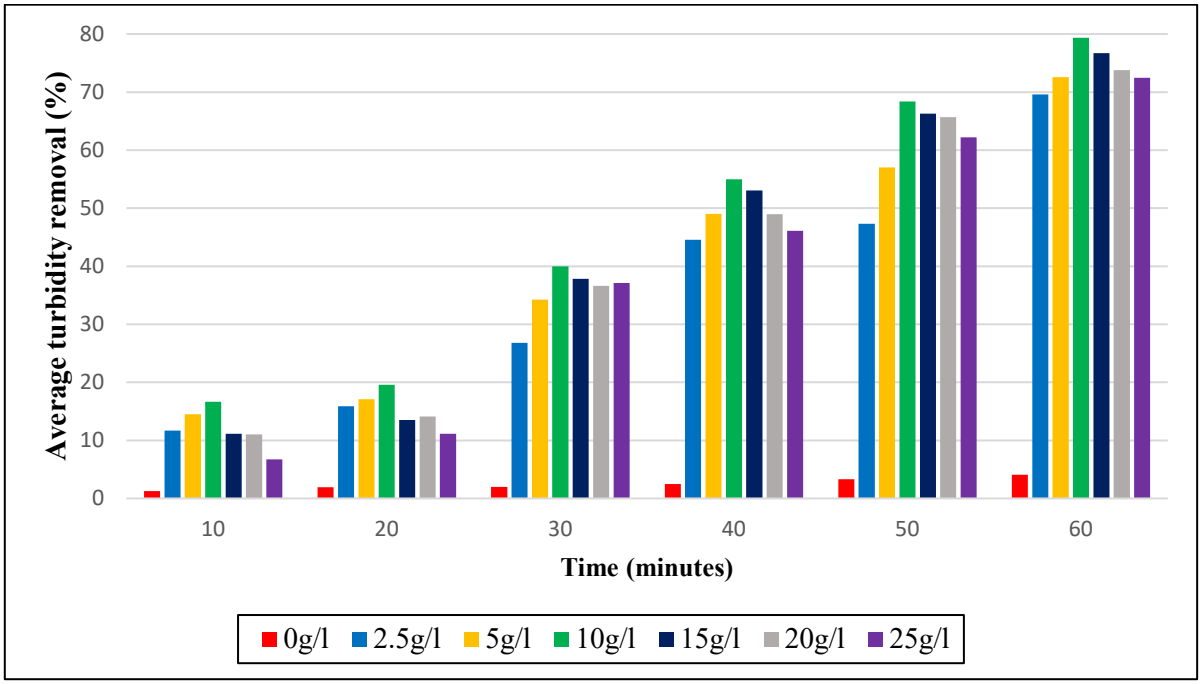


Figure 41: The effect of the dose of salt-extracted heterogeneous coagulant derived from *Cicer arietinum* on turbidity removal (from an initial turbidity of 200 ± 0.5 NTU) over 60 minutes

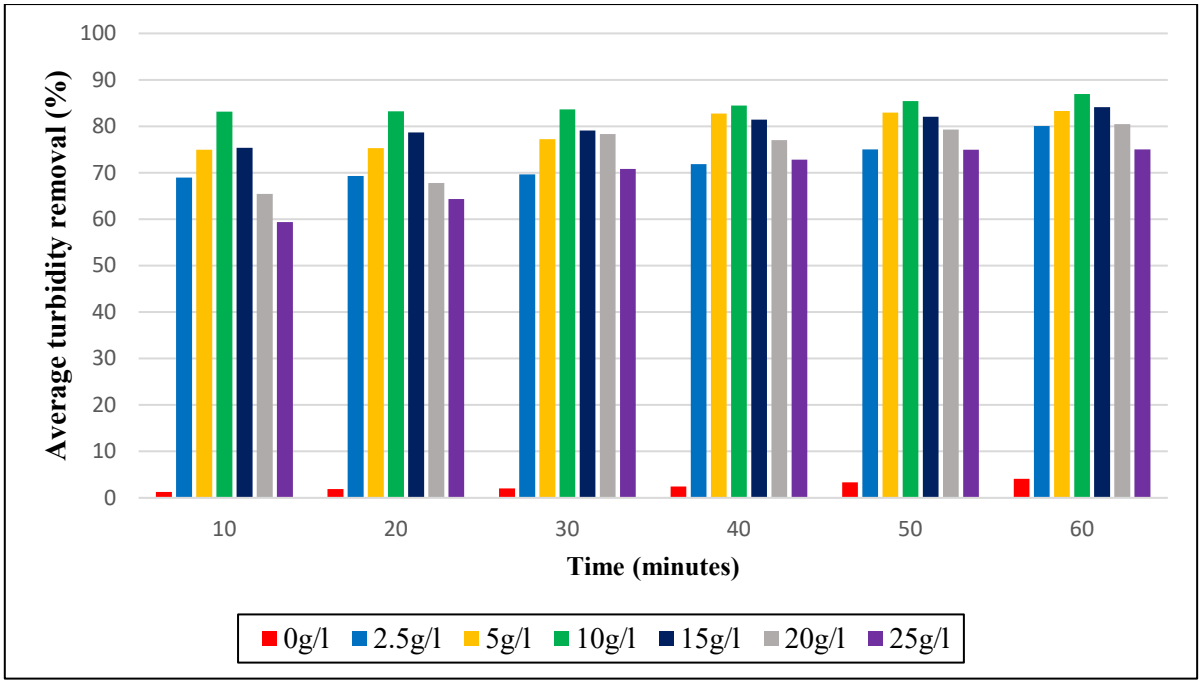


Figure 42: The effect of the dose of salt-extracted heterogeneous coagulant derived from *Glycine max* on turbidity removal (from an initial turbidity of 200 ± 0.5 NTU) over 60 minutes

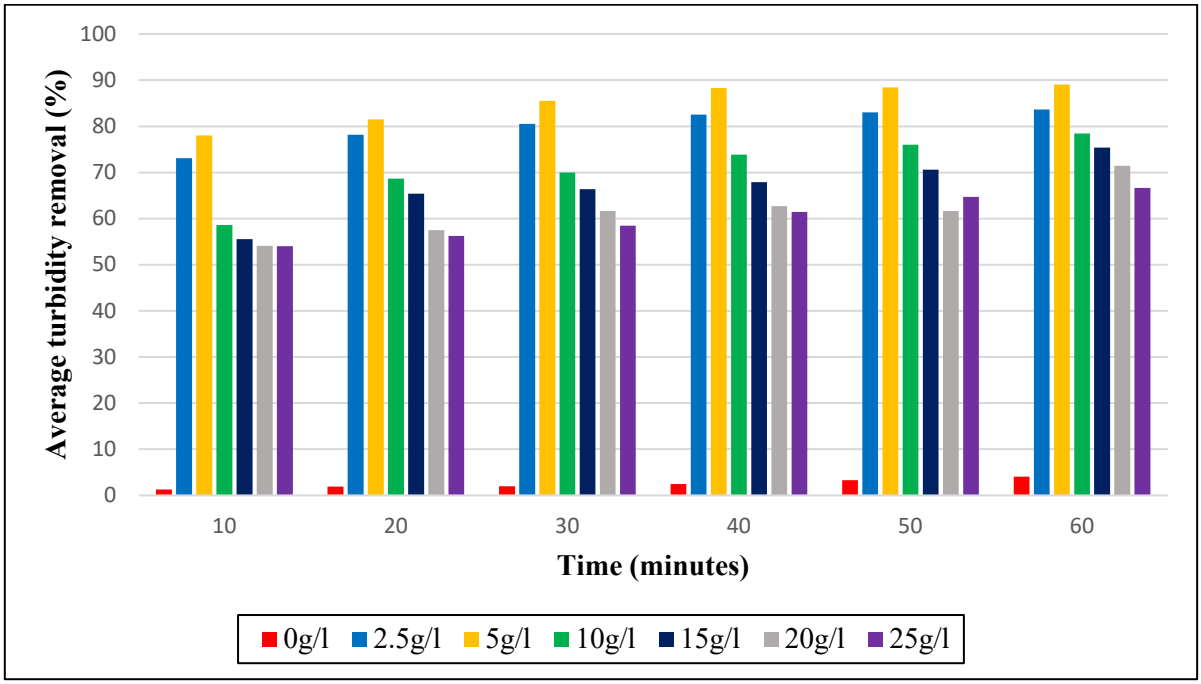


Figure 43: The effect of the dose of salt-extracted heterogeneous coagulant derived from *Vigna mungo* on turbidity removal (from an initial turbidity of 200 ± 0.5 NTU) over 60 minutes

Three runs of turbidity testing at the optimum coagulant dose were performed to test repeatability. Table 22 provides the average turbidity removal percentage of each plant species at the optimum coagulant dose. The results indicate that *Vigna mungo* achieved the highest average turbidity removal percentage (89.6%) at the lowest optimum coagulant dose (5g/l) and *Cicer arietinum* attained the lowest average turbidity removal percentage of 80.4% at the highest coagulant dose (10g/l). Relatively low standard deviations were noted in turbidity measurements indicating excellent reproducibility.

Table 22: Average turbidity removal (from an initial turbidity of 200 ± 0.5 NTU) at the optimum dose of salt extracted heterogeneous coagulating agent for four plant species after 60 minutes of sedimentation

Plant species	Optimum dose (g/l)	Average turbidity removal (%) ±standard deviation
Vigna mungo	5	89.6±2.637
Glycine max	10	87.4±2.741
Vigna unguiculata	5	83.1±2.262
Cicer arietinum	10	80.4±1.443

4.3.2. Aqueously extracted heterogeneous coagulant

The performance of the aqueously extracted heterogeneous coagulant product in turbidity removal at different doses was evaluated using the jar test. The jar test was carried out for the aqueously extracted heterogeneous coagulant in a similar manner to the tests involving the salt-extracted heterogeneous coagulating agents. From the first iteration, which was carried out over a wide range of coagulant doses (0.1-100g/l), it was established that maximum turbidity removal could be achieved in a dose range of 2.5-25g/l. The second iteration, depicted in Figures 44 to 47, show the average turbidity removal percentage (from an initial turbidity of 200 ± 0.5 NTU) at different coagulant doses over a 60-minute period. It is important to note that three readings of residual turbidity were taken, and the average turbidity removal is shown in Figures 44 to 47.

Figures 44 to 47 show an increase in turbidity removal with time. In general, turbidity removal increases with increasing coagulant dose up to a maximum point and thereafter decreases with increasing coagulant dose. Figures 44 and 45 show that at a dose of 15g/l, maximum average turbidity removal percentages of 79.5% and 76.6% were achieved in tests involving *Vigna unguiculata* and *Cicer arietinum* respectively. A high dose of 20g/l of salt-extracted heterogeneous coagulant derived from *Glycine max* was required to achieve a relatively low maximum turbidity removal of 77.6% (shown in Figure 46). At 10g/l, tests involving *Vigna mungo* show maximum turbidity removal of 80.2% as seen in Figure 47.

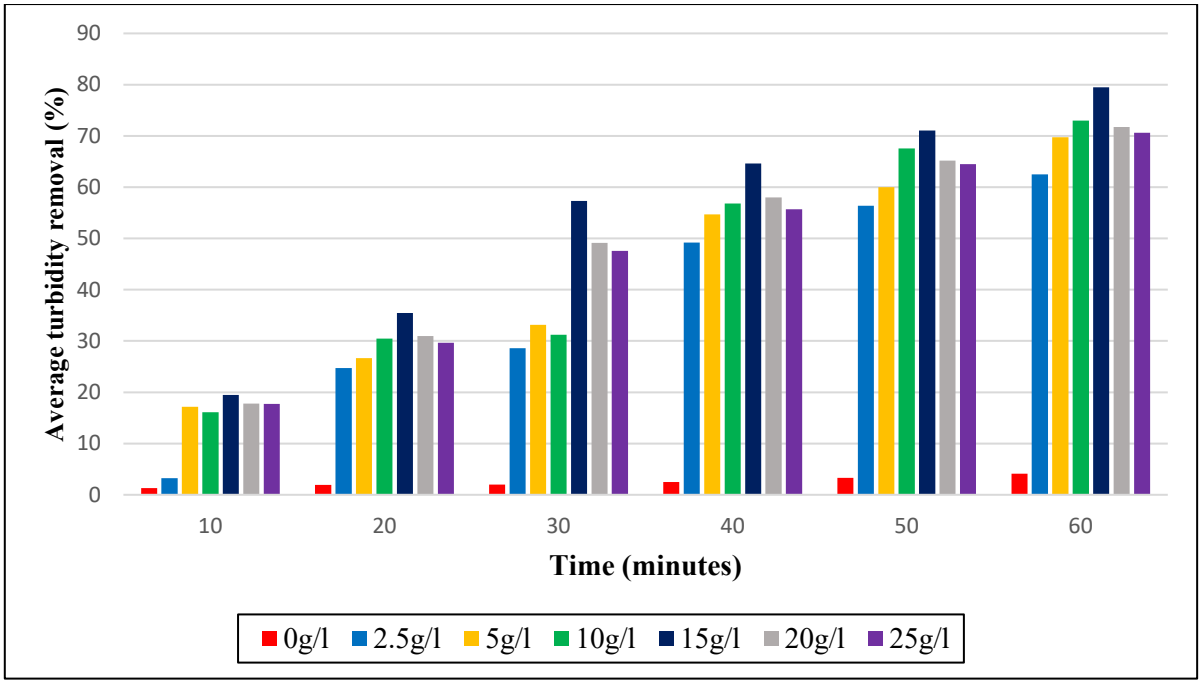


Figure 44: The effect of the dose of aqueously extracted heterogeneous coagulant derived from *Vigna unguiculata* on turbidity removal (from an initial turbidity of 200 ± 0.5 NTU) over 60 minutes

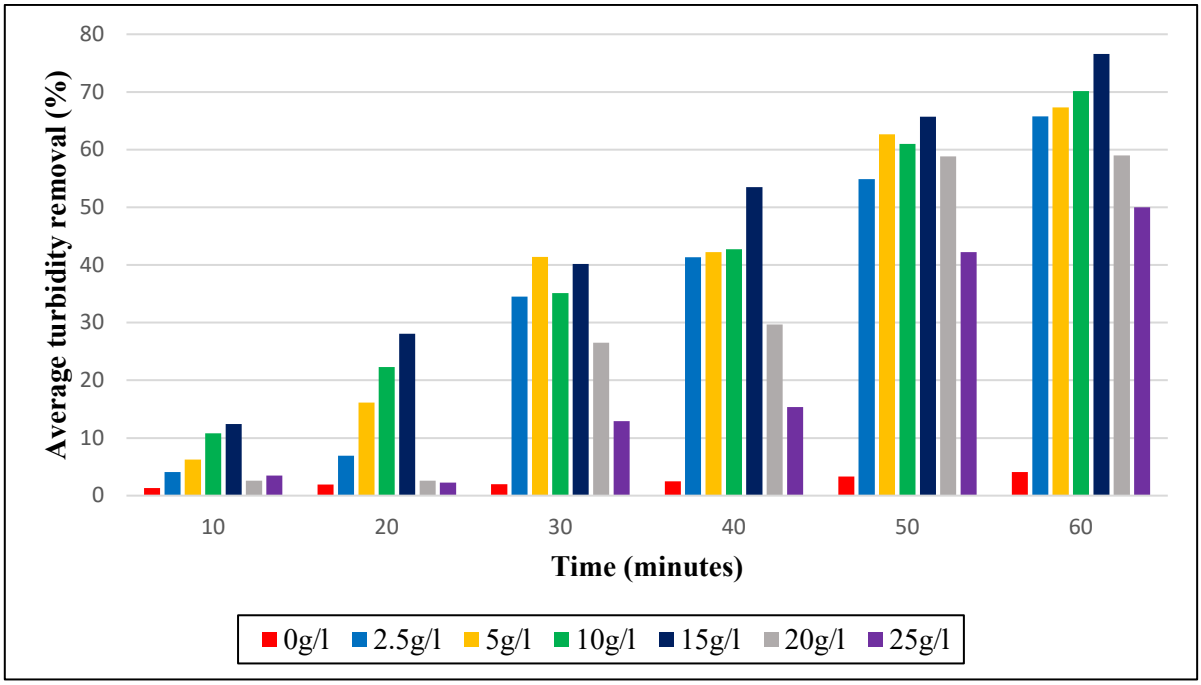


Figure 45: The effect of the dose of aqueously extracted heterogeneous coagulant derived from *Cicer arietinum* on turbidity removal (from an initial turbidity of 200 ± 0.5 NTU) over 60 minutes

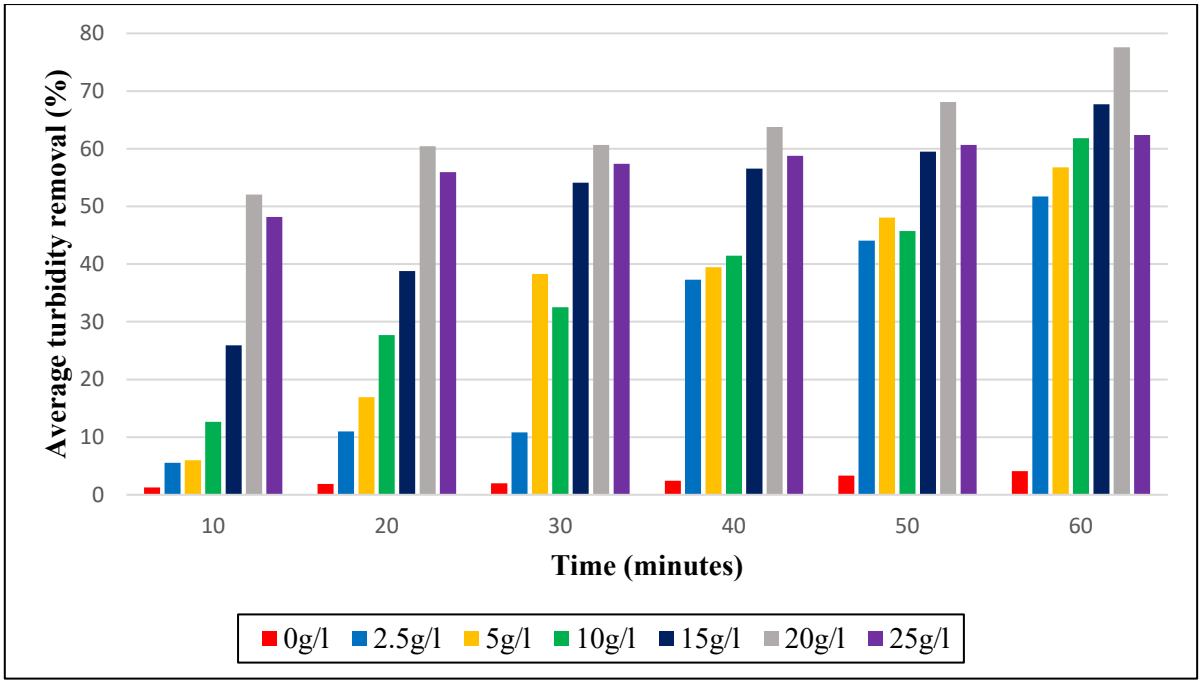


Figure 46: The effect of the dose of aqueously extracted heterogeneous coagulant derived from *Glycine max* on turbidity removal (from an initial turbidity of 200 ± 0.5 NTU) over 60 minutes

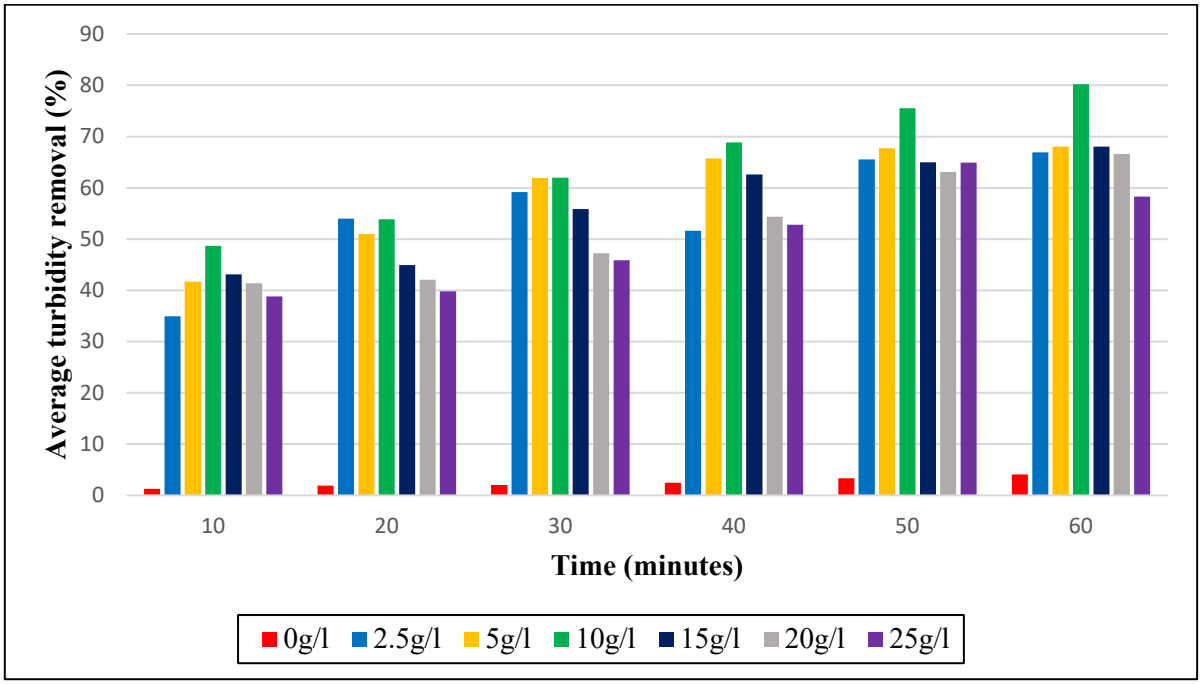


Figure 47: The effect of the dose of aqueously extracted heterogeneous coagulant derived from *Vigna mungo* on turbidity removal (from an initial turbidity of 200 ± 0.5 NTU) over 60 minutes

The jar test was performed three times using the optimum aqueously extracted coagulant dose (as determined by iteration 2). The results indicated in Table 23 show that the highest average turbidity removal percentage of 82.3% was achieved at the lowest optimum dose of 10g/l for *Vigna mungo*. The lowest turbidity removal (78.2%) was noted for *Cicer arietinum* at an optimum dose of 15g/l. The highest optimum dose of 20g/l was required for *Glycine max* to achieve a maximum turbidity removal of 79.1%. The low standard deviations indication good repeatability.

Table 23: Average turbidity removal (from an initial turbidity of 200 ± 0.5 NTU) at the optimum dose of aqueously extracted heterogeneous coagulating agent for four plant species after 60 minutes of sedimentation

Plant species	Optimum dose (g/l)	Average turbidity removal (%) ±standard deviation
Vigna mungo	10	82.3±1.987
Glycine max	20	79.1±1.082
Vigna unguiculata	15	80±1.437
Cicer arietinum	15	78.2±1.179

4.3.3. Heterogeneous coagulant and coagulants studied in literature

The turbidity removal performance of the aqueously extracted heterogeneous coagulants produced in this study and the coagulants developed and tested in literature are summarised in Table 24. Note that the performance of *Vigna mungo* and *Moringa oleifera* in turbidity removal from an initial turbidity of 200 NTU could not be sourced from literature, therefore their performance in water with initial turbidities of 482 NTU and 236 NTU respectively, was used for comparison. The aqueously extracted homogeneous plant-derived coagulants and aluminium sulphate coagulant studied in literature achieved high turbidity removal percentages (>95.9%). In this study, the aqueously extracted heterogeneous coagulants, attained turbidity removal percentages in the range of 78.2% to 80%. However, no direct comparisons can be made between the results obtained in literature and that attained in this study due to the differences in extraction parameters(such as extraction time), and jar test parameters (such as mixing speed and time and sedimentation time) as shown in Table 24.

Table 24: Processing conditions and performance of the heterogeneous coagulant and coagulants studied in literature

Coagulant	Extraction time (mins)	Jar test conditions						Turbidity removal (%)
		Initial turbidity (NTU)	Rapid mixing		Slow mixing		Sedimentation	
			Speed (rpm)	Time (mins)	Speed (rpm)	Time (mins)	Time (mins)	
Aqueously extracted homogeneous plant-derived coagulants								
Vigna mungo (Mbogo, 2008)	10	482	100	1	40	15	30	98.8
Glycine max (Hussain & Haydar, 2020)	30	200	200	1	30	25	30	93.4
Vigna unguiculata (Hussain & Haydar, 2019)	30	200	200	1	30	25	30	96.8
Cicer arietinum (Jaramillo & Epalza, 2019)	60	200	150	2	45	25	30	98
Moringa oleifera (Awad, et al., 2013)	30	236	150	1	40	7.5	30	95.9
Aqueously extracted heterogeneous plant-derived coagulants								
Vigna mungo	45	200	125	1	40	15	60	82.3
Glycine max	45	200	125	1	40	15	60	79.1
Vigna unguiculata	45	200	125	1	40	15	60	80
Cicer arietinum	45	200	125	1	40	15	60	78.2
Inorganic coagulants								
Aluminium sulphate (Jaramillo & Epalza, 2019)	-	200	150	2	45	25	30	97.5

CHAPTER 5: DISCUSSION

5.1. Extraction of active components

Extractability is a measure of the quantity of active components from the raw material that is solubilised into the extraction solvent under specific conditions. The extractability of protein into solution is dictated by its solubility, which depends on the interaction between the protein and the solvent. Under optimal extraction conditions, proteins experience a net positive or negative charge which enhances the repulsive forces between the proteins. This in turn favours either protein solubilisation or protein stability in dispersion (Hui, 1996).

5.1.1. Salt extraction

To investigate the effect of salt concentration on protein extractability, salt extraction of the active components present in the seeds of the four plant species under investigation was performed. Literature indicated that salt extraction is capable of greatly improving the extraction of the proteins from plant components. This is especially true for the legume proteins, found in the selected plants, since these proteins constitute approximately 70% globulin proteins (Karaca, et al., 2011). Globulins are soluble in salt-water solutions, therefore extraction of these proteins using salt-extraction would recover more protein as opposed to other extraction methods. In addition, salt extraction was employed as it does not require an extreme acidic or alkaline pH and does need an elevated temperature. Salt extraction typically occurs at a natural pH level of the protein-water-salt mixture which is 5.5- 6.5 (Lam, et al., 2018).

Several factors need to be considered when selecting a salt for the purpose of extraction. Factors such as chemical inertness, ion stability, environmental impact, toxicity, cost and availability are key considerations in salt selection (Hyde, et al., 2017). Sodium chloride (NaCl) was utilised for salt extraction as it has been found to greatly improve extractability of legume proteins (Liu & Hung, 1998). Further, it is low-cost, non-toxic and readily available, making it suitable for application in rural communities.

When salts are added to a solution containing proteins, the salts screen the electric double-layer (which is made up of a stern and diffuse layer) surrounding the protein molecule. This in turn lowers the zeta potential of the protein as well as the amount of electrostatic repulsive forces acting on the protein. The reduced net charge then causes the proteins to aggregate through hydrophobic interactions, Van der Waals attractive forces and hydrogen bonding. Protein precipitation thereafter occurs when the aggregates are at an appropriate size and number. Salting-in occurs when salts encourage protein-water interactions and the ordering of the hydration layers surrounding the protein molecule, which in turn increases protein solubility. Salting-out takes place when salts encourage ion-water interactions that disrupt the hydration layers surrounding the protein molecule causing the hydrophobic components to be exposed, which results in protein aggregation and precipitation (Lam, et al., 2018).

5.1.1.1 The optimum salting-in concentration

The salting-in process was employed to increase the solubility of the plant protein in solution for protein extraction. According to Maurer, et al. (2011), protein solubility should increase with increasing salt concentration up to a maximum solubility, and thereafter decrease with increasing salt concentration. The point at which the protein has the highest solubility in solution is deemed as the optimum salting-in concentration.

Since proteins have unique solubility curves, each plant protein should have a unique optimum salting-in concentration, at which most proteins are extracted into solution. In order to determine the optimum salting-in concentration for each plant protein, the proteins were firstly extracted at varying sodium chloride concentrations (in the range of 0M to 0.9M NaCl). This range was selected as the salting-in of proteins typically takes place at low ionic strengths, corresponding to salt concentrations ranging from 0.1M to 1M (Lam, et al., 2018). This is evidenced by literature where excellent extractability was achieved for *Cicer arietinum, Vigna unguiculata, Vigna mungo* and *Glycine max* at salt concentrations of 0.4M (Osman, et al., 2005), 0.6M (Marobhe, et al., 2007), 0.5M (Sasikala & Muthuraman, 2017) and 0.3M (Yuan, et al., 2002) respectively.

In order to maximise protein extractability in saline solutions, the optimum salting-in concentration was determined via the ammonium precipitation method. The ammonium sulphate used in this process has the ability to salt-out proteins as a co-precipitate due to the saturation concentration providing a relatively high molarity that enables the precipitation of most proteins (Nehete, et al., 2013). The ammonium precipitation method is typically employed as a protein fractionation process, where different proteins can be precipitated at different ammonium sulphate concentrations. However, since protein separation was not the focus of this study, all proteins were precipitated through the gradual addition of ammonium sulphate until its saturation concentration.

Since ammonium sulphate causes the extracted proteins to aggregate and form a precipitate, the amount of solid residue produced after ammonium precipitation of the filtered protein-rich solution was used as an indication of the amount of proteins extracted. A high mass of precipitate formed from the protein-rich solution indicated a high protein content in the solution, hence high extractability, and vice versa. Therefore, the degree of extractability at different salt concentrations could be established.

Figure 29 showed that in general, the mass of precipitate (hence extractability) increases with increasing salt concentration up to a point and thereafter decreases. This was characteristic of the salting-in phenomenon. The salting-in phenomenon occurs because of thermodynamic linkage between the free energy of salt binding and protein solubility. Due to the small ionic radii of organic anions, they can achieve closer proximity and bind more strongly to the proteins than inorganic cations.

As the sodium chloride concentration increases, the salt dissociates, and the chloride anions bind to the proteins, which increases the overall negative charge on the protein. The increase in solubility could be due to more anionic chloride ions, rather than sodium cations, binding to the protein, which increases its negative charge and induces solubility.

At higher salt concentrations (beyond the optimum salting-in concentration), the sodium cations could bind with the negatively charged protein, neutralise charged groups and reduce its overall negative charge which results in lowered protein solubility (Liu & Hung, 1998). Note that the fixed, charged sites on the protein, that were available for ions to selectively and strongly bind to, are limited. Therefore, at high salt concentrations, all the charged sites on the protein become filled which leads to the excess chloride ions competing with protein for free water, causing in salting-out (Hui, 1996).

The extractability of *Vigna unguiculata* active components increases with increasing sodium chloride concentration up to 0.4M and thereafter decreases as seen in Figures 29 and 30. Therefore, 0.4M is deemed as the optimum salting-in concentration for *Vigna unguiculata* active component extraction, which corresponds to the findings of Ahmed, et al. (2012). The curve in Figure 29 flattens after the optimum salting-in concentration indicating that ionic strength has no significant effect on extraction after the optimum salting-in concentration. Similarly, there was an initial increase in the extractability of *Glycine max* proteins with an increase in salt concentration (as shown in Figure 29); this correlates to the findings of Yuan, et al. (2002). From Figures 29 and 32, it can be viewed that the highest extractability of *Glycine max* protein was achieved at a salt concentration of 0.5M. This was deemed as the optimum concentration for salting-in and correlates to the salt concentration for maximum *Glycine max* protein(especially β-congylycinin) extraction in literature (Deak, et al., 2006). The optimum salting-in concentration of *Glycine max* was higher than that of *Vigna mungo* and *Vigna unguiculata*, possibly because the salt content of *Glycine max* is approximately 2mg/100g (Nutrition Value, 2020), which is the lowest of all the plant species studied(as indicated in Table 17). Therefore, greater salt addition was necessary to facilitate optimum salting-in.

Cicer arietinum shows a similar trend to *Vigna unguiculata* however, a much higher salting-in concentration of 0.8M is required to maximise protein extractability as viewed in Figures 29 and 33. This is higher than the optimum salting-in concentration of 0.4M reported by Osman, et al. (2005), however the seed properties (in terms of protein and salt content) could have varied depending on factors such as seed maturity. In Figure 29, it can be viewed that the *Cicer arietinum* curve flattens after the optimum salting-in concentration, indicating that salt concentration does not significantly affect extractability after the optimum concentration.

The results for the extraction from *Vigna mungo* seeds are depicted in Figures 29 and 33. Similar to the other plant species, the extractability of active components increases with increasing salt concentration, up to a salt concentration of 0.3M, and decreases thereafter. The peak of the curve was at a relatively low salt concentration and could be attributed to the high salt content of the seed(38mg/100g) (Nutrition Value, 2020). Since the salt concentration in solution was initially high due to the presence of salt from the seed, an excessive amount of additional salt was not necessary to induce optimal extraction. However, the mass of precipitate produced decreases relatively significantly after the optimum salting-in concentration which indicates that the high salt content may have caused rapid salting-out.

From Figure 29 it can be observed that *Glycine max* produced the greatest amount of precipitate. This could be because *Glycine max* has the highest protein content (40%) (Badole, et al., 2015) and a high globulin content (46.5%) (Ciabotti, et al., 2016), which makes more proteins available for extraction. *Vigna mungo* produces the second highest amount precipitate (at low ionic strengths) compared to the other plant species. This may be due to *Vigna mungo* having a high protein content (28.4%) (Padhye, 1978) and the highest globulin fraction (81%) (Padhye, 1978), making more salt-soluble proteins available for extraction. Compared to *Vigna mungo*, *Cicer arietinum* produced a greater amount of precipitate at salt concentrations greater than approximately 0.45M, possibly because *Vigna Mungo* reached its peak much sooner and undergoes salting-out at concentrations greater than 0.45M, while *Cicer arietinum* still experiences salting-in and only reaches its peak at a concentration of 0.8M. *Vigna unguiculata* had the lowest extractability in salt solutions even though its globulin protein content was relatively high. Low protein solubility can be attributed to the formation of ionic bonds within and between adjacent proteins which could have led to protein aggregate formation (Osman, et al., 2005).

5.1.2. Aqueous extraction

Aqueous extraction of the four plant species under investigation was performed by dispersing the comminuted seeds in deionized water via vigorous agitation. Solid-liquid separation was also carried out to separate the protein-rich filtrate from the solid residue. Aqueous extraction was investigated in this study due to its simplicity and therefore easy application in developing nations that do not have access to specialised chemicals and equipment. Further, it can be remotely performed, and the method poses no safety hazard to the user or the environment.

An excellent solvent is characterised by its optimum extraction capacity and its ability to maintain the stability of the chemical structure of the compounds that it extracts. Water is a good solvent due to its polarity which allows it to electrostatically interact (via charge-based attractions) with other polar molecules and ions. It is an environmentally friendly extractant as it can be used and disposed without negatively impacting the environment. It is also non-toxic and low-cost making it an ideal candidate for protein extraction (Mihaylova & Lante, 2019).

The extraction of proteins in water involves two types of interactions: interactions between the protein and water and interactions among the proteins (Hui, 1996). Protein-protein interactions are facilitated by hydrophobic interactions which leads to the precipitation of the proteins. Protein-water interactions however promote protein hydration and solubility. These protein-water interactions, and thereby protein solubility, are affected by environmental factors (such as temperature, pH and ionic strength) and by processing methods (such as extraction).

Ammonium precipitation was carried out to quantify protein extractability since ammonium sulphate causes extracted proteins to aggregate. Thus, a higher mass of precipitate formed from the filtered protein-rich solution indicated higher extractability of proteins into solution and vice versa. The results shown in Table 16 indicated that aqueous extraction of *Vigna mungo* produced the most amount of precipitate (3.103g), and *Glycine max* produced the second highest amount of precipitate (2.612g). It was anticipated that *Glycine max* would have produced the greatest amount of precipitate when aqueously extracted since the seed had the highest protein content (40%) (Badole, et al., 2015) and the highest albumin content (31.8%) (Ciabotti, et al., 2016), which would have made a greater amount of water-soluble proteins available for extraction compared to other plant species. However, the higher extractability of *Vigna mungo* proteins could be attributed to the higher sodium content of the seed (38mg/100g) (Nutrition Value, 2020) compared to the other plant species(shown in Table 17). The high salt content could have induced salt-extraction within the seed, which could have led to greater extractability(since globulin extraction would be favoured) (Sefa-Dedeh & Stanley, 1979).

From Table 17, it can be viewed that *Vigna unguiculata* has a greater protein content (31%) (Lim, 2012) and albumin content (24.9%) (Agustin, et al., 2020) than *Cicer arietinum,* which has a protein and albumin content of 28.9% (Chang, et al., 2012) and 12% (Chang, et al., 2012) respectively, therefore it was anticipated that protein extractability from *Vigna unguiculata* seeds would be higher than *Cicer arietinum*. However, the greater amount of precipitate formed by aqueous extraction of *Cicer arietinum* could be due to the high sodium content (24mg/100g) (Nutrition Value, 2020) in the seed which may have induced salt extraction as in the case of *Vigna mungo*.

5.1.3. Comparison between the performance of aqueous extraction and salt extraction

Legume proteins consist of globulin and albumin proteins. Since albumin is a water-soluble protein, aqueous extraction would recover high yields of this protein. However since albumin proteins make up approximately 10-20% of the total protein content, while globulins constitute 70% of the protein content (Karaca, et al., 2011), it is anticipated that extraction methods that favour globulin recovery(such as salt extraction) would recover more protein overall.

Salt extraction (at the optimum salting-in concentration) produced a greater amount of precipitate than aqueous extraction for all plant species under investigation as shown in Figure 29. This could be attributed to the solubility properties of the proteins contained in each seed. Each plant species has a higher globulin content than albumin content. Since globulin is more soluble in salt solutions, more proteins (which are mostly globulins) are readily extracted via salt-extraction.

From Figure 29 and Tables 15 and 16, it is important to note that the mass of precipitate formed by aqueous extraction of the active components of *Vigna mungo* was 3.103g, which was comparable to that formed at its optimum salting-in concentration (3.513g) and was higher than the mass of precipitate formed at salt concentrations in the range of 0.5M to 0.9M. This could be attributed to a high mineral content (and subsequent salt content) of *Vigna mungo* which may have induced salt extraction that generally favours globulin protein extraction. Since globulin protein is the major storage protein found in *Vigna mungo,* significant amounts of protein were extracted via salt extraction.

5.2. Protein loading

The surface of porous adsorbents (such as silica) has complex geometrical structures that have varying affinities for molecules. Therefore, adsorption directly correlates to the interactions between the adsorbent and adsorbate (Kumar, et al., 2011). Some proteins' tendency to adsorb to surfaces can be attributed to the nature of its amino acid side chain which is present on the protein surface. Some of these amino acids can be apolar and are generally found buried inside the protein, whereas some amino acids can be polar and charged and tend to exist on the protein surface. The electrostatic interaction between the charged amino acid side chain and an oppositely charged adsorbent surface leads to a change in the free energy and favours adsorption.

Electrostatic interactions generally facilitate adsorption of proteins onto hydrophilic/charged surfaces. It was theorised that conformationally stable proteins can only adsorb onto charged surfaces via electrostatic attractive conditions. However, more recent studies have shown that proteins with the same net charge as the adsorbent can still adsorb strongly. This is anticipated to be the reason why all the seed proteins which have net negative charges at a neutral pH (due to their pI being below the neutral pH of the solution as seen in Table 20), still adsorb onto the negatively charged silica surface.

Adsorption at the wrong side of the isoelectric point can be attributed to a possible patchy charge distribution on the protein, where the protein has both negative and positive patches on its surface. Under this assumption, the protein orients itself in a way that allows the positively charged patch to attractively interact with the negatively charged adsorbent through the release of counter-ions (Meissner, et al., 2015).

Another plausible reason for adsorption at the wrong side of the isoelectric point may be due to the difference in pH between the micro-environment of the adsorbent and the bulk solution which encourages electrostatic interactions via the Donnan effect. Using this theory, the negatively charged proteins would have a more positive charge near the surface than in the bulk solution.

Another interesting observation from the analysis carried out by Mathe, et al. (2013), was that the net charge of the protein was not the only governing factor in adsorption. The study suggests that the total number of positively and negatively charged amino acids contribute to polarity, density and hydrophilicity. Consequently, a high number of positively charged clusters(due to the number of positively charged amino acids) may help initiate local electrostatic interactions with the negatively charged silica surface(particularly the SiO^- groups). It can be viewed in Table 21, that each plant species has relatively high quantities of positively charged amino acids which could been involved in localised electrostatic interactions with the negatively charged silica substrate. Particularly, it was found that arginine content was present in the highest quantities(in terms of positively charged amino acids) and this amino acid tends to have a higher probability of electrostatically interacting with silica than histidine and lysine (Mathe, et al., 2013).

5.2.1. Salt-extracted heterogeneous coagulating agent

Salting-out extraction is applied for its ability to facilitate the recovery of organic compounds in a simple manner that does not result in significant changes to the existing batch process. Thus, salting-out has been extensively used for protein isolation by ammonium sulphate precipitation, protein crystallisation, and in analytical chemistry for the extraction of analytes from biological materials (Hyde, et al., 2017).

Salt concentrations beyond the optimum salting-in concentration were selected and applied for each plant species to determine the optimum salting-out concentration and investigate the effects of ionic strength on protein loading. Salting-in was utilised to extract proteins into solution by increasing its solubility. In order to facilitate loading of the proteins onto a silica substrate, the solubility of the proteins need to decrease. By increasing the salt concentration to the optimum salting-out concentration, the resulting extensive protein particle aggregation is anticipated to improve adsorption (Xu, et al., 2015).

Hydrophobic patches constitute approximately 48-70% of the solvent-accessible surface of proteins. Hydrogen-bonded water molecules (which are dynamically similar to the bulk water molecules) that bridge polar sites usually dissect the large hydrophobic patches. Salt present in solution is able to remove the water molecules that are bound to the protein. When the salt concentration is low, the ions are hydrated by bulk water molecules. However, when the salt concentration is increased, the greater ion hydration requirement leads to the salt utilising the weakly bound water molecules from the hydrophobic patches on the protein, which thereby reduces the proteins solubility and induces salting-out (Meng, et al., 2013).

Increasing the salt concentration beyond the optimum salting-in concentration was investigated for potential improvement in protein loading efficiency. Following extraction, the salt concentration was increased beyond the optimum salting-in concentration for each plant species to investigate the optimal salting-out concentration for this purpose. Ammonium precipitation was utilised as an indication of adsorption efficiency, where the least amount of precipitate produced from the filtrate was indicative of the greatest amount of protein adsorbed onto silica (which was present in the retentate). Therefore, the optimum salting-out concentration was deemed as the concentration that precipitated the least amount of proteins.

The preferential interaction theory states that proteins prefer to be hydrated, thus hydrophobic patches on the protein are generally covered by ordered water molecules. When salt is added to the solution, it causes the positive charge of the protein chemical potential. If the salt concentration is increased to an optimum salting-out concentration, the chemical potential of the hydrophobic patches on the protein will be high enough to interact with the substrate (Meng, et al., 2013).

Figures 34 to 38 show changes in precipitate formation with changing salt concentration. This indicates that adsorption still occurs even when the proteins and the substrate both have net negative charges (since these proteins and silica have a pI that is below the neutral pH of the solution as indicated in Table 20) and should be electrostatically repulsed. However, adsorption can still occur via hydrogen bonding, and hydrophobic and lateral interactions since protein adsorption can be induced by electrostatic and non-electrostatic interactions (Pham, et al., 2020). Adsorption could also be due to positively charged patches on the protein surface interacting with the negatively charged silica or due to the Donnan effect.

In general, an initial increase in adsorption is expected with increasing salt concentration up to an optimum salting-out concentration, where adsorption is maximised. Positively charged amino acids present in the seeds of the four plant species were expected to interact with the negatively charged adsorbent surface through localised electrostatic interactions. However, negatively charged amino acids (such as glutamic acid and aspartic acid) are present in higher quantities than positively charged amino acids in the seed protein as viewed in Table 21. These negatively charged amino acids would experience electrostatic repulsion interactions with the silica surface. However, it is possible that through sodium chloride addition, the Na^+ ions at least partially screen the negatively charged silica surface which could also promote the adsorption of negatively charged amino acids, in certain places, onto the surface which would lead to enhanced overall adsorption (Mathe, et al., 2013).

Once the optimum salting-out concentration is reached, a general decline in adsorption with increasing salt concentration occurs. At high salt concentrations, it is possible that proteins adsorb onto the silica surface beyond monolayer coverage. In multilayer adsorption, a single layer of proteins is adsorbed via the patch-controlled mechanism. Subsequent layers form through protein-protein electrostatic interactions among the adsorbed proteins and the proteins still in solution. This causes the protein layer to grow (linearly or exponentially) with an increase in protein concentration (Nfor, et al., 2010).

Weak adsorption has been observed beyond monolayer coverage, especially if the isoelectric point of the protein is close to the pH of the solution (Meissner, et al., 2015). Multilayer adsorption was reported for the adsorption of the cationic protein *Moringa oleifera* onto a silica substrate (Kwaambwa, et al., 2010) and could be a reason for decreases in adsorption of the plant proteins used in this study. Another reason for the decline in adsorption could be attributed to net charge reversal that can occur when cationic proteins adsorb onto negatively charged silica surfaces (Nordmark, et al., 2018).

As indicated by the mass of precipitate formed, the adsorption of *Cicer arietinum* onto the silica substrate appeared to increase with increasing salt concentration beyond the optimum salting-in concentration (0.8M),and is maximised at the optimum salting-out concentration (0.825M) as seen in Figure 36.

Similarly, in Figure 37, it can be viewed that *Glycine max* adsorption onto silica was greatly improved when the salt concentration was increased from the optimum salting-in concentration of 0.5M to the optimum salting-out concentration of 0.6M. Beyond the optimum salting-out concentration, adsorption appears to decrease with increasing salt concentration as seen in Figure 37. The lowered adsorption can be attributed to multilayer adsorption beyond this point. Multilayer adsorption of *Glycine max* proteins, as reported by Salas, et al. (2012), was attributed to disulphide linkages in β-conglycinin which encourage the association of glycinin at the adsorbent surface. The reduced adsorption at high sodium chloride concentrations could be due to alteration of the net charge on the substrate due to the interaction with the salt ions (Janhom, 2010).

A minimal change in adsorption with increasing salt concentration in the range of 0.9-1.3M for *Cicer arietinum* and 0.7-1M for *Glycine max* was noted in Figure 34. This indicates that adsorption of the proteins of these plant species does not depend heavily on salt concentration at very high ionic strengths. A high occupation of binding sites at high salt concentrations could have led to a limited adsorption.

As seen in the Figure 34, *Vigna mungo* showed minimal changes in adsorption from 0.3M to 0.6M. This indicates that in this salt concentration region, attractive protein-substrate interactions are nearly balanced by repulsive protein-protein interactions. Both interactions are screened by salt therefore, adsorption in this region is only weakly influenced by salt (Meissner, et al., 2015). However, adsorption changes drastically in the salt concentration range of 0.6M to 0.8M, where it maximises at an optimum salting-out concentration of 0.75M(as seen in Figure 38). The negatively charged groups in and on the surface of the protein molecules experience electrostatic repulsion. At higher ionic strengths, these repulsive electrostatic interactions on the protein and substrate are screened which consequently results in greater amounts of proteins being adsorbed at the optimum salting-out concentration.

Vigna unguiculata protein adsorption was found to decrease with increasing ionic strength beyond the optimum salting-in concentration of 0.4M as shown in Figure 34 and 35. This reversal of the influence of salt was observed in a study by Meissner, et al. (2015), where the reduction in adsorption at high salt concentrations was attributed to the competition of non-electrostatic and electrostatic contributions to the adsorption energy. The reduced adsorption at higher salt concentrations could also be due the complexity of the protein molecule, surface roughness of the seed and/or the orientation of the adsorbed protein on the silica surface (Salas, et al., 2012). Therefore, salting-out was not ideal for *Vigna unguiculata* as it did not lead to improved adsorption efficiency.

It was assumed that multi-protein adsorption occurs because proteins were not separated and purified prior to loading. In multi-protein adsorption, one species is adsorbed via transport-limited adsorption until the point where the adsorbent surface appears to be full of at least one species. Thereafter, these initially adsorbed species are displaced by other (more surface active) species which preferably adsorb onto the surface (Wertz & Santore, 1999).

The quantities of surface-active proteins extracted from each plant species could have resulted in differences in adsorption. Differences in adsorption could also be due to differences in hydrophobic patch distribution on the protein surface, protein conformation and/or intermolecular interactions between adsorbed proteins (which could be influenced by salt concentration) (Meng, et al., 2013).

For *Glycine max*, it was noted that the protein is readily salted-out at a relatively low salt concentration of 0.6M which may indicate that there may have been a significant amount of hydrophobic side chains on the surface of the protein molecule which were capable of interactions, which lead to aggregation if either the protein net charge was changed or the solvent surface tension was increased(due to salt addition). The hydrophobic side chains would have then been unavailable for intrachain stabilisation and compaction of the protein molecule (Martenson, 1978).

When proteins exist in solution, electrostatic repulsion interactions between them are weak as they are adequately distanced from each other. At low salt concentrations it was noted by Meng, et al. (2013), that electrostatic repulsion interactions exist among protein molecules. When proteins are adsorbed onto a substrate, electrostatic repulsions between the free protein molecules in solution and the adsorbed protein molecules prevents the adsorption of the free protein molecules onto the substrate. At higher salt concentrations, this electrostatic repulsion among protein molecules is weakened and hydrophobic interactions between the protein and substrate are enhanced and takes precedence, leading to increased adsorption of free protein molecules. This explains the large difference in adsorption from the optimum salting-in concentration (0.3M) to the optimum salting-out concentration (0.75M) for *Vigna mungo*.

5.2.2. Aqueously extracted heterogeneous coagulating agent

It was anticipated that adsorption would correlate to the amount of proteins extracted aqueously. Using the results shown in Table 16, this would mean that protein adsorption affinity of each plant species would be arranged as *Vigna mungo* > *Glycine max* > *Cicer arietinum* > *Vigna unguiculata*. However, almost the opposite trend was found in this study as shown in Table 19. From Table 19, it can be viewed that in the absence of salt, *Cicer arietinum* produced the least amount of precipitate (5.919g), indicating the greatest adsorption. In comparison, *Vigna mungo* produced the most precipitate (7.126g) and therefore had lower adsorption levels. It is possible that adsorption correlates directly to the number of positively charged amino acids present in the seed since *Cicer arietinum* has the highest positively charged amino acid content (18.6%) and *Vigna mungo* has a lower positively charged amino acid content (15.9%) as indicated in Table 21. The greater amount of positively charged amino acids could have led to increased interaction with the electronegative silica surface hence leading to increased adsorption. *Glycine max* had lower protein adsorption than *Vigna unguiculata* and *Cicer arietinum* which could be attributed to the low salt content present in the *Glycine max* seed(2mg/100g) (Nutrition Value, 2020) compared to the other plant species. The higher salt content of the seeds of *Vigna unguiculata* and *Cicer arietinum* (as indicated in Table 17) could have enhanced adsorption.

5.2.3. Comparison between performance of aqueously extracted and salt-extracted heterogeneous coagulating agent

For *Glycine max*, as shown in Figure 34, it was noted that at 0M NaCl, the highest mass of precipitate was formed, indicating poorest adsorption in the absence of salt. Basic polypeptides exist inside the *Glycine max* protein molecule due to its composition being mostly hydrophobic amino acid units. An increase in ionic strength encourages the exposure of basic polypeptides on the surface proteins. At high ionic strengths, the water becomes a poor solvent for the polypeptides (due to their hydrophobic nature). Consequently, hydrophobic interactions at the substrate surface are encouraged which results in higher adsorption than that experienced in only distilled water. Further, at high ionic strengths, the protein layers adsorb more rigidly onto the substrate surface since the proteins carry less bound water (Salas, et al., 2012).

Similar trends to *Glycine max* were viewed for *Cicer arietinum* (in Figure 34), where the lowest adsorption was experienced at 0M. However, the amount of precipitate formed at 0M NaCl did not differ greatly from the amount of precipitate formed at salt concentrations above 0.9M. This could be attributed to the high sodium content (24mg/100g) (Nutrition Value, 2020) present in the *Cicer arietinum* seed which could have enhanced adsorption, even in the absence of additional salt. Improved adsorption in the presence of salt, for *Glycine max* and *Cicer arietinum*, could also be due to the greater amount of proteins present in solution, that were preferably extracted by salt extraction rather than aqueous extraction

In Figure 34, *Vigna mungo* showcases a similar trend to *Cicer arietinum* in terms of having similar adsorptions at 0M and in the salt concentration range of 0.3M-0.6M. This can be explained by the high salt content(38mg/100g) (Nutrition Value, 2020) of *Vigna mungo* which could have enhanced adsorption even when no salt was added to the solution. For *Vigna unguiculata*, increasing the salt concentration beyond 0.5M reduces the adsorption of proteins. At these high ionic strengths, lower protein adsorption was experienced compared to the adsorption at 0M and 0.4M. This can be explained by possible multilayer adsorption that could have occurred which may have led to the adsorbed protein layer growing unfavourably with an increase in protein concentration that is expected to be present in solutions with a higher NaCl concentration.

5.3. Turbidity removal testing

Turbidity refers to the cloudiness or haziness of a body of fluid that is generally caused by the presence of many particles that are usually invisible to the naked eye. High turbidity levels are indicative of the presence of a high number of individual particles in the water. If highly turbid water is consumed, it poses greater risks of the development of gastrointestinal diseases since contaminants (like bacteria and viruses) can become attached to the suspended solids. It is therefore crucial that turbidity levels be brought down to acceptable levels (<5 NTU) prior to consumption (Maheswari, et al., 2019).

In this study, turbidity was used as an indication of the amount of colloidal material in the water, therefore it was a means of measuring water quality and assessing the effectiveness of the coagulating agent in turbidity removal. Stock solutions of synthetic water were prepared by the addition of 10 grams of kaolin to 1 litre of water. The samples were allowed 24 hours to settle prior to testing to ensure complete hydration of the kaolin particles (Choudhary & Neogi, 2017).

To determine the amount of stock solution to add to 200ml of distilled water to achieve the desired turbidity level, different volumes of stock solution were added to 200ml of distilled water and the turbidity was measured and recorded. The average turbidity measurements at each volume addition had low standard deviations indicating good repeatability. Through linear regression, a relationship between volume of stock solution and turbidity was established. From Figure 39, it can be viewed that turbidity increases linearly with stock solution dose. A strong positive correlation between these variables is justified by the high R^2 value of 0.9922. From Figure 39, it was determined that approximately 10ml of stock solution needs to be added to 200ml of distilled water to achieve a turbidity of 200 NTU.

A fixed turbidity level of 200 NTU was utilised for turbidity testing to simulate natural water sources that have a similar turbidity level. The turbidity of untreated water sources (such as rivers) can be 200 NTU (Feria-Díaz, et al., 2016), however the turbidity of these sources may exceed 200 NTU based on variations in factors such as the time of collection, weather and surrounding environment (Keogh, et al., 2017). The initial turbidity of 200 NTU was also selected to simulate medium turbid waters typically utilised in literature (Miller, et al., 2008), such that comparisons can be drawn between the results in this study and that found in literature. The jar test method was used to measure the turbidity removal effectiveness and involved the addition of a specific quantity of heterogeneous coagulant to a turbid water sample. Thereafter, rapid mixing (at 125rpm for 1 minute), slow mixing (at 40rpm for 15 minutes) and sedimentation (for 60 minutes) was carried out. Rapid mixing was essential to maximise collisions between the coagulant and turbid water particles, while slow mixing was necessary to maximise floc growth. Slow mixing and rapid mixing velocity were not increased beyond 60rpm and 160rpm respectively to prevent re-dispersion of the formed flocs. Sedimentation allows for the formed flocs to settle, and thereafter be easily removed (Yani, et al., 2019).

It is possible that the heterogeneous coagulant has positive and negative charges on its surface as in the study by Nordmark, et al. (2018), which involved the adsorption of cationic *Moringa oleifera* proteins onto a silica substrate. Since the cationic proteins do not occupy all sites on the silica surface, in practice, the silica surface will still have patches of negative charges. The positive charges can be attributed to the protein attached on the surface. Since the heterogeneous coagulant is anticipated to have both positive and negative charges, both anionic and cationic particulates from suspension can be removed from the water through electrostatic attraction, making the heterogeneous coagulant dual functional (Nfor, et al., 2010).

Floc formation occurs because the coagulant charge neutralises the charge on the turbid water particles and promotes particle aggregation. The properties of the floc, such as the floc size and strength are important considerations in the coagulation process. Smaller particles tend to have lower removal efficiencies after flocculation because the smaller size of the particles results in them settling slower than larger particles that have a similar density. If flocs are exposed to sheer forces, they tend to rupture. Following rupture, the flocs' surface charge may change, and they may become partially re-stabilised which makes regrowth difficult. Therefore, it is imperative to ensure floc properties are suitable to enable easy removal in later processes (Ma, et al., 2012). The process of floc formation is highly dependent on the dosage of coagulant; therefore, it was imperative to determine the optimum coagulant dose in order to reduce dosing cost, mitigate sludge formation and optimise turbidity removal performance (Patel & Vashi, 2012). Coagulant underdosing may result in low energy between particles thereby producing weak and small flocs. However, overdosing may cause charge reversal resulting in poor coagulation performance (Yani, et al., 2019)

In order to establish the optimum dose of the heterogeneous coagulant, testing was initially done for aqueously and salt-extracted heterogeneous coagulants over a wide range of doses (0.1-100g/l). Floc formation was determined, through visual experimental observation, at doses higher than 2.5g/l and the turbidity measured increased with increasing coagulant doses at doses greater than 25g/l, therefore testing was carried out in a narrower dose range of 2.5g/l to 25g/l.

5.3.1. Optimum dose of salt-extracted heterogeneous coagulant

The optimum dose was determined to ensure maximum turbidity removal, minimise coagulant wastage and mitigate the negative impacts to the treated water due to improper dosing. In the second iteration of turbidity testing, coagulant doses of each plant species were varied from 2.5 to 25g/l and added to a turbid water sample that had an initial turbidity of 200 NTU. Following rapid and slow mixing, turbidity measurements were made every 10 minutes (for 60 minutes) in the sedimentation period.

Figure 40 to 43 show the change in turbidity removal with increasing dose of salt-extracted heterogeneous coagulants derived from the four plant species under investigation. For all plant species, the general trend was that turbidity removal increases with increasing coagulant dose and thereafter decreases (or remains relatively constant). The increase in turbidity removal efficiency in all cases occurs because as more coagulant is added, more turbid water particles could be destabilised by charge neutralisation and efficient collisions, which promotes increased aggregation (floc formation) (Ma, et al., 2012). The decrease in turbidity removal efficiency can be due to coagulant overdosing which causes charge reversal, resulting in the turbid particles undergoing re-dispersion and re-stabilisation (Yani, et al., 2019). The decrease could also be due to the aggregation or overlapping of adsorption sites at high doses, which may have led to a reduced adsorbent surface area and increased path length available for adsorption of the colloidal particles (Bello, et al., 2017).

The point at which maximum turbidity removal was achieved was determined as the optimum coagulant dose. The results in Table 22 indicate that the optimum dose of a salt-extracted heterogeneous coagulant derived from *Vigna unguiculata*, *Cicer arietinum, Glycine max* and *Vigna mungo* was 5g/l, 10g/l, 10g/l and 5g/l respectively. The average turbidity removal achieved at these doses were 89.6%, 80.4%, 87.4% and 89.6% respectively. Thus, the heterogeneous coagulant derived from *Vigna mungo* performed the best in comparison to the other coagulants, as it achieved the highest turbidity removal at the lowest dose. In contrast, *Cicer arietinum* attained the lowest turbidity removal at the highest dose of 10g/l.

Although the optimum dose of each coagulant fell within a narrow range (5-10g/l), the differences in turbidity removal efficiency achieved for each coagulant could be attributed to differences in extraction and loading efficiency of each plant species as well as the differences in coagulation activity of the active components. Different coagulants destabilise particles at different degrees. Generally, if the counter-ion has a higher valence, then the coagulant's destabilising effect will be greater. Therefore, a lower coagulant dose will be required (Patel & Vashi, 2012).

It was noteworthy that turbidity removal efficiency did not change significantly with time, especially at the optimum dose, in the case of *Glycine max* and *Vigna mungo* (as seen in Figure 42 and 43), which indicates that a long sedimentation time will not be required to achieve high turbidity removal efficiencies. However, tests involving *Vigna unguiculata* and *Cicer arietinum* require sufficient sedimentation time to achieve a good turbidity removal efficiency (as seen by the fairly significant change in turbidity removal with time shown in Figures 40 and 41). These differences are possibly due to the differences in floc settling properties.

Upon comparison of the performance of each heterogeneous coagulant to natural settling in turbidity removal, it can be viewed in Figures 40 to 43 that the heterogeneous coagulant had a significantly higher turbidity removal efficiency and rate. In 60 minutes, natural settling could only remove a maximum of 4.1% of turbidity whereas the coagulants could achieve substantially greater turbidity removal efficiencies in the range of 80.4-89.1%. This indicated that the salt-extracted heterogeneous product was a viable solution in expediting turbidity removal.

5.3.2. Optimum dose of aqueously extracted heterogeneous coagulant

Figures 44 to 47 show the results of the investigation into the optimum dose of aqueously extracted heterogeneous coagulants derived from *Vigna unguiculata*, *Cicer arietinum, Glycine max* and *Vigna mungo* respectively. The general trend for all plant species was that turbidity removal efficiencies increase with increasing coagulant dose. Once the maximum turbidity removal is achieved at the optimum dose, the turbidity removal decreases with increasing coagulant dose.

The increase in turbidity removal, with dose, can be attributed to higher levels of charge neutralisation (and floc formation). In addition, there is greater availability of adsorption sites for the attachment of colloidal particles from suspension, therefore more particles are effectively removed (Adekola, et al., 2014). Charge reversal or the overlapping of adsorption sites may have led to the decrease in turbidity removal efficiency at higher doses.

As indicated in Table 23, the optimum dose and corresponding average maximum turbidity removal efficiency determined in the jar tests involving *Vigna unguiculata*, *Cicer arietinum, Glycine max* and *Vigna mungo* were 15g/l and 80%, 15g/l and 78.2%, 20g/l and 79.1%, and 10g/l and 82.3% respectively. These results indicate that the salt-extracted heterogeneous coagulant derived from *Vigna mungo* performed the best as it had the highest turbidity removal at the lowest dose. *Glycine max* did not perform as well in these tests as the other plant species since it required the highest dose to achieve a turbidity level that was the third highest (however it was very close to the removal percentage of salt extracted *Cicer arietinum*). These differences in turbidity removal efficiency and doses can be attributed to the coagulating properties of the plant species. In addition, the extractability and adsorption affinity of the proteins play a key role in turbidity removal performance.

The results shown in Figure 45 for the heterogeneous coagulant derived from *Cicer arietinum* indicate that turbidity removal increases significantly with time. Low turbidity removal efficiencies were noted after 10 minutes of sedimentation (which were comparable to that of natural settling). Thus, sufficient time needs to be allowed for the formation of flocs in order to achieve high turbidity removal rates. It is also worthwhile investigating if turbidity removal increases further beyond the 60-minute sedimentation time allowed for in this study.

Similar results are noted from Figure 44 for *Vigna unguiculata* tests, indicating that a sufficiently long sedimentation period is necessary to achieve high turbidity removal. An interesting observation was that the turbidity removal percentages do not change significantly with increasing dose in the 40 to 60-minute range, thus indicating that if adequate sedimentation time is allowed, then a slightly lower dose of coagulant can be utilised to achieve good turbidity removal efficiencies.

Figures 46 and 47show the results from the tests involving *Glycine max* and *Vigna mungo* respectively and indicate that turbidity removal efficiency does not change significantly with time when the optimum dose of coagulant is used. Thus, water treatment can be performed quickly if there are time limitations. In addition, the results for *Vigna mungo* (shown in Figure 47) indicate that turbidity removal efficiencies at low doses were comparable to that at the optimum dose (especially in the first 30 minutes of sedimentation), thus lower doses can be utilised to achieve similar turbidity removal efficiencies if coagulant availability is limited.

The aqueously extracted heterogeneous coagulants were able to achieve much higher turbidity removal efficiencies and rates than that achieved when particles were allowed to settle naturally. This is evidenced for all plant species, for which maximum turbidity removal rates were between 78.2% and 82.3% after 60 minutes of settling, whereas natural settling could only achieve a maximum turbidity removal of 4.1% after 60 minutes of sedimentation. These results thus showcase the effectiveness of the aqueously extracted heterogeneous coagulant in turbidity removal.

5.3.3. Comparison between performance of aqueous extracted and salt extracted heterogeneous coagulant

It was noted from Table 22 and 23 that salt-extracted heterogeneous coagulating agents could achieve higher turbidity removal at lower doses than aqueous extraction for all the plant species under investigation. This improvement is especially visible in the results of *Glycine max,* where the coagulant dose (which was 20g/l of aqueously extracted heterogeneous coagulant) was halved to 10g/l when the salting-in and salting-out process was employed. The corresponding turbidity removal is also higher (87.4% compared to 79.1% achieved by the aqueously extracted heterogeneous coagulant) if salting-in and salting-out is incorporated into the coagulant development process. Although a relatively small improvement in turbidity removal (when using salt-extracted heterogeneous coagulant compared to aqueous heterogeneous coagulant) was achieved in tests involving *Vigna unguiculata* and *Cicer arietinum*, the required doses were lowered. In the case of *Vigna unguiculata*, the optimum dose of salt-extracted heterogeneous coagulant was three times less than the optimum dose of aqueously extracted heterogeneous coagulant.

The differences in the performance of the salt-extracted heterogeneous coagulant and the aqueously extracted heterogeneous coagulant could be due to the variability in the amount of plant-derived active components that were available for interaction with the turbid particles in solution. The salt-extracted heterogeneous coagulating agent was anticipated to have a greater quantity of plant-derived proteins due to the higher extractability and adsorption of the proteins onto the silica substrate in the presence of salt, as determined in the extraction and loading results section of this study. Thus, the salt-extracted heterogeneous product had a greater quantity of active material and hence adsorption sites available to induce floc formation and improve turbidity removal.

Overall, the results indicate that employing the salting-in and salting-out process in the development of plant-derived heterogeneous coagulants may be beneficial as higher turbidity removal efficiencies can be achieved at lower doses which makes it a viable option for water treatment processes

5.3.4. Comparison of heterogeneous coagulant turbidity removal performance to that of coagulants studied in literature

The aqueously extracted heterogeneous coagulants developed in this study achieved high turbidity removal percentages in the range of 78.2-82.3% as shown in Table 24. In literature, aqueously extracted homogeneous coagulants derived from *Glycine max, Vigna unguiculata* and *Cicer arietinum* showcased high turbidity removal percentages (>95.9%). The differences between the performance of coagulants studied in literature and the coagulants produced in this study could be due to the variation in processing speed and time for extraction, mixing and sedimentation (in the jar test). Since the amount of protein contained in plant material typically varies, the amount of protein available for extraction could have also been different in each study. Extraction efficiency could have also influenced coagulant performance in both cases, however there was no evidence of this as the precise amount of active components present in the coagulant products was not quantified. Loading efficiency (in the case of the heterogeneous coagulant) could have also impacted turbidity removal results. Thus, no direct comparisons can be drawn between the homogeneous coagulants (studied in literature) and the heterogeneous coagulants (produced in this study) at this stage. Similarly, the differences in the processing conditions used in literature, for *Moringa oleifera* and *Aluminium sulphate* coagulants, and in this study, for the heterogeneous coagulants, prevent a direct comparison from being made.

However, it must be noted that the use of homogeneous coagulants for water treatment has been found to increase the organic content in water and thereby promote micro-organism growth. These homogeneous coagulants also produce flocs with poor settling characteristics. Therefore, the heterogeneous coagulant was preferred not only for its high turbidity removal performance(especially when the salting-in and salting-out process is incorporated) but also for its ability to mitigate the occurrence of organic matter in treated water and produce flocs with improved settling characteristics. In addition, the plant-derived heterogeneous coagulants were preferred (as opposed to chemical coagulants) due to its non-toxicity, biodegradability and low cost.

5.4. Adaptation of methodology for rural communities

There is a lack of water treatment systems, especially in rural areas, therefore a simple and cost-effective point-of-use technology (using coagulation) can be utilised for front-end water treatment. The plant species under investigation have the potential to achieve satisfactory turbidity removal efficiencies. Further, the methodology used in this study can be easily adapted for use in rural and peri-urban areas.

It is imperative that communities have access to a rudimentary treatment protocol to enable easy and effective application. It should be advised that although natural coagulants can have the potential to significantly reduce turbidity levels, they may not be able to ensure complete pathogen elimination and an additional low-cost treatment system should be employed if possible (Lea, 2014).

The coagulant doses established in this study can be used as a guideline for the treatment protocol. However, due to the expected variance in raw water conditions and dose performance, communities can carry out a simple jar test to establish the optimal dosage for specific water conditions. This jar test would involve the addition of the natural coagulants in varying doses to turbid water samples followed by rapidly mixing, gentle stirring and sedimentation. The optimum coagulant dose can be established by examining the colour and turbidity level of each sample.

A simple procedure, using common household items, is provided by Lea (2014) for the use of *Moringa oleifera* in rural communities and can be adapted to the plant species used in this study. The procedure involves grinding the seeds using a mortar or grinding stone, sieving through a tea strainer, adding the powder to a small quantity of previously clarified water, vigorously shaking for a few minutes and thereafter allowing the solution to sediment. The solution can be then filtered in a tea strainer (and/or muslin cloth). The extracted active components can then be loaded onto a substrate such as silica (or beach sand). This would involve the addition of silica to the protein-rich solution, mixing and sieving through a tea strainer. The heterogeneous coagulant can then be added to the turbid water. Rapid mixing, slow mixing and settling can then take place followed by filtering and boiling prior to consumption.

Simple technologies and indigenous species that can be easily procured, can be utilised to reduce operational costs and overcome limitations such as shortages in skilled personnel. In order to carry out rapid mixing, the static mixer can be employed and floc formation and settling can be induced by the baffled pond. If turbidity levels after coagulation processes are still above acceptable levels for drinking(>5 NTU), then sand filtration or pebble matrix filtration can be employed in addition to coagulation (Kristianto, 2017). A flowchart for the use of natural coagulants in rural communities is provided in Figure 48.

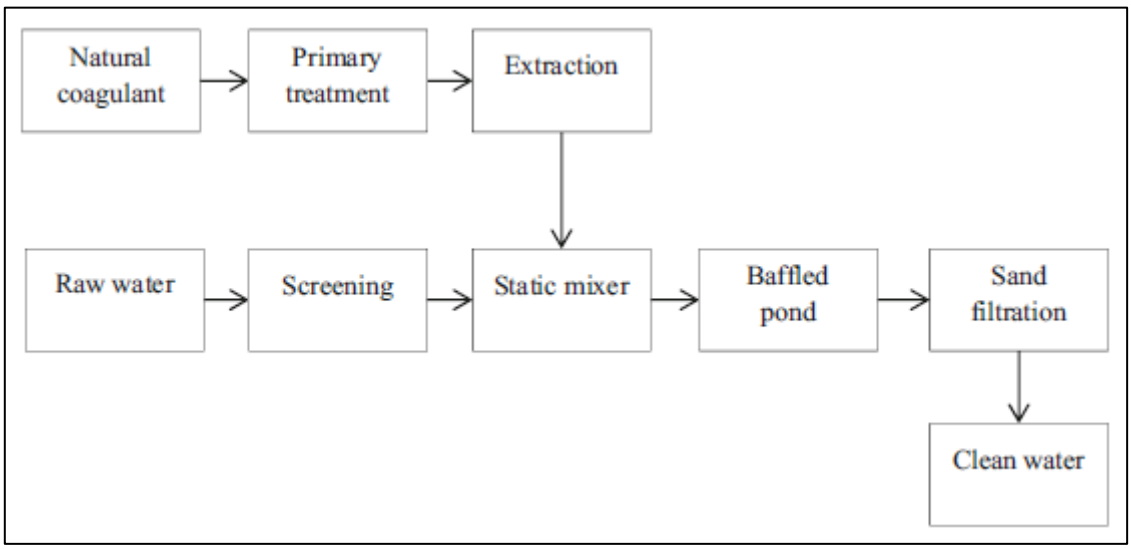


Figure 48: Water treatment process using natural coagulants for rural areas (Kristianto, 2017)

5.5. Impact assessment

In remote and/or developing nations, many communities are forced to consume water that contains sediment and harmful contaminants (like faeces) due to the lack of access to purifying agents and knowledge on how to properly treat water prior to consumption. In addition, in many developing countries, women and children have to travel far distances to collect clean water. To combat these issues and have a positive societal impact, this study was designed to provide a means of water treatment that would allow for safe treatment and consumption practices and protect the health and well-being of people. Since the treatment process can be conveniently applied in any location, it saves time and effort that can be better spent elsewhere. The use of natural coagulants derived from plant materials can be a social-change factor since these plants are widely available and easy to store.

Natural coagulants produce lower volumes of sludge (which is biodegradable in nature) compared to conventional coagulants, therefore there are lower costs associated with the handling and disposal of sludge. Following treatment, the sludge produced can be employed as a bio-fertiliser/bio-compost. However, sludge must be properly treated and disposed to avoid bacterial contamination in the treated water. Care must also be taken to prevent an increase in organic matter and chemical oxygen demand (COD) in the treated water (Sasikala & Muthuraman, 2017).

The use of heterogeneous coagulating agents offers several benefits such as improved turbidity removal performance. In addition, it has been shown that rinsing the silica with sodium chloride solutions following water treatment has the ability to desorb proteins and enable re-use of the silica substrate (Nordmark, et al., 2018). Further, heterogeneous coagulants reduce the occurrence of organic matter, which is prevalent when homogeneous coagulants are used. This mitigates the negative environmental impacts associated with the presence of organic matter in water, such as the depletion of oxygen in water bodies, eutrophication and the death of aquatic life.

The method of water treatment proposed in this study offers the benefit of being simple, low-cost and easily modifiable. Although this study focuses on only four plant species, the method of testing can be readily adapted for indigenous plants or other easily accessible plant species. Technologically, the protein extraction, loading and coagulation process used in this study was much less complex than conventional processes and does not require specialised equipment and maintenance. Further, pH adjustment is not necessary (as in conventional treatment) therefore specialised chemicals are not required for this purpose.

CHAPTER 6: CONCLUSIONS

A major challenge currently faced by many countries is the lack of access to clean and safe drinking water. Due to a lack of knowledge of the water treatment process and resources (such as treatment agents), many communities consume unsafe water that contains sediment and pathogens. Consequently, outbreaks of waterborne diseases (such as cholera) are becoming more prevalent. Therefore, this study was aimed at establishing a simplistic and cost-effective method of decentralised front-end water treatment via the development of a heterogeneous coagulant derived from plant components.

The conclusions derived from this investigation are:

- Aqueous and salt-extraction were effective in recovering the active components from the seeds of the four plant species under investigation.
- In general, extractability of the active components was found to increase with increasing salt concentration up to a maximum point and thereafter decrease with increasing salt concentration, which was characteristic of the salting-in and salting-out phenomenon.
- Active components from *Vigna unguiculata, Cicer arietinum, Glycine max* and *Vigna mungo* were optimally extracted at salting-in concentrations of 0.4M, 0.8M, 0.5M and 0.3M respectively.
- Salt-extraction generally allowed for greater extractability than aqueous extraction due to the high globulin content of the plant species under investigation.
- In the case of *Vigna mungo*, the high sodium content of the seed enabled high protein extractability even when aqueous extraction was employed.
- The salting-out process was found to improve the loading efficiency of proteins extracted from *Cicer arietinum, Glycine max* and *Vigna mungo*.
- In general, loading efficiency was found to increase with increasing salt concentration up to a maximum point and thereafter decrease with increasing salt concentration.
- *Vigna unguiculata* exhibited reduced adsorption with increasing salt concentration beyond its optimum salting-in concentration, therefore salting-out was not ideal for this plant species.
- The optimum salting-out concentration was determined to be 0.4M, 0.825M, 0.6M and 0.75M for *Vigna unguiculata, Cicer arietinum, Glycine max* and *Vigna mungo* respectively.
- Turbidity removal was found to generally increase with increasing coagulant dose and decrease or remain relatively constant beyond the optimum dose (at which turbidity removal is maximised).
- The optimum dose and maximum turbidity removal achieved was 15g/l and 80%, 15g/l and 78.2%, 20g/l and 79.1% and 10g/l and 82.3% using aqueously extracted heterogeneous coagulants derived from *Vigna unguiculata, Cicer arietinum, Glycine max* and *Vigna mungo* respectively.

- When the salting-in and salting-out process was employed in extraction and loading, 5g/l and 89.6%, 10g/l and 80.4%, 10g/l and 87.4%, and 5g/l and 89.6% was the optimum dose and maximum turbidity removal achieved using heterogeneous coagulants derived from *Vigna unguiculata, Cicer arietinum, Glycine max* and *Vigna mungo* respectively.
- Extraction, loading and turbidity removal when using the salting-in and salting-out process in the development of the heterogeneous coagulant was found to be more effective than carrying out these processes in the absence of salt.
- Maximum turbidity removal due to natural settling was determined to be 4.1% and was considerably lower than that achieved through the use of a coagulant.
- Overall, *Vigna mungo* was established as the best candidate for the development of a heterogeneous coagulant due to its excellent performance in turbidity removal at low doses and relatively low salt concentration requirements for high extraction and loading efficiencies.

CHAPTER 7: RECOMMENDATIONS

Specific recommendations that would be worthwhile investigating in future studies are:

- The testing of other plant species (especially indigenous plant species such as (*Hibiscus sabdariffa*) as potential candidates for the development of a heterogeneous coagulating agent can be performed.
- Protein adsorption onto the silica substrate can be studied in greater detail, using methods such as neutron reflection, to aid adsorption modelling, in order to gain a greater understanding of the behaviour of the plant proteins at the silica interface.
- Turbidity removal testing can be conducted using raw water collected from different natural water sources, such as rivers and streams, to investigate the effectiveness of the heterogeneous coagulants in real-life applications.
- The effects of natural coagulants on other water quality parameters such as pH, colour, total dissolved solids, and total organic carbon can be studied.

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Appendix A: Raw data and calculation summary

The raw data and calculation summary sample for *Cicer arietinum* is presented in Appendix A.

A.1. Extraction of active components

Iteration 1 of the aqueous and salt extraction process for *Cicer arietinum* is shown in this section. Sample 1 contains the aqueous extraction medium (0M NaCl) and samples 2 to 6 contain salt extraction mediums of varying concentrations (0.1M to 0.9M) as depicted in Table 25. These samples were prepared to determine the optimum salting-in concentration for extraction.

Table 25: Desired and actual parameter values used in the preparation of the aqueous and salt extraction mediums (with concentrations ranging from 0.1M to 0.9M) and the mass of raw *Cicer arietinum* biomass added to the extraction medium in iteration 1

		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Desired NaCl molarity	**mol/l**	0	0.1	0.3	0.5	0.7	0.9
Volume of distilled water	**l**	0.04	0.04	0.04	0.04	0.04	0.04
Desired mols of NaCl to add	**mol**	0	0.004	0.012	0.02	0.028	0.036
Desired mass of NaCl to add	**g**	0	0.234	0.701	1.169	1.636	2.104
Actual mass of NaCl added	**g**	0	0.226	0.702	1.159	1.638	2.116
Actual NaCl concentration	**mol/l**	0	0.097	0.3	0.496	0.7	0.905
Desired mass of biomass to add	**g**	2	2	2	2	2	2
Actual mass of biomass added	**g**	2.002	2	2	1.999	2.001	2.001

To enable the gradual change in ammonium saturation up to100% ammonium sulphate saturation, different masses of ammonium sulphate were added to each sample to achieve the desired ammonium sulphate saturation percentage as indicated in Table 26 and 27. This was necessary to facilitate the precipitation step in extraction. The mass of precipitate produced by ammonium precipitation of the extracted proteins is shown in Table 28.

Table 26: Mass of ammonium sulphate to be added to a 1000ml solution and a 40ml solution to achieve the desired ammonium saturation percentage

Saturation interval (%)	Mass of ammonium sulphate to 1000ml solution (g)	Mass of ammonium sulphate to 40ml solution (g)
0-20	114	4.56
20-40	123	4.92
40-60	132	5.28
60-80	143	5.72
80-100	157	6.28

Table 27: The volume of the contents of each sample following extraction of *Cicer arietinum* active components and the actual mass of ammonium sulphate added to samples 1 to 6 to achieve the desired ammonium saturation percentage (iteration 1)

		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Volume after NaCl extraction	**ml**	40	40	40	40	40	40
Saturation interval (%)		**Mass of ammonium sulphate added**					
0-20	**g**	4.564	4.562	4.56	4.563	4.56	4.559
20-40	**g**	4.924	4.922	4.927	4.917	4.921	4.926
40-60	**g**	5.276	5.286	5.286	5.278	5.284	5.283
60-80	**g**	5.718	5.721	5.72	5.716	5.721	5.727
80-100	**g**	6.279	6.279	6.284	6.286	6.276	6.282

Table 28: Mass of precipitate formed following extraction of *Cicer arietinum* active components, ammonium precipitation, filtration and drying (iteration 1)

		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Mass of filter paper and watch glass	**g**	41.236	42	37.983	37.967	41.339	41.811
Mass of filter paper, watch glass and precipitate after drying	**g**	42.627	44.3	40.675	41.08	44.585	45.041
Mass of precipitate	**g**	1.391	2.3	2.692	3.113	3.246	3.23

A.2. Loading of active components

Iteration 1 of the aqueous and salt loading of *Cicer arietinum* protein is shown in this section. Table 29 shows the extraction parameters for aqueous extraction (sample1) and optimum salt-extraction at a NaCl concentration of 0.8M for *Cicer arietinum* (samples 2 to 7). From Table 29, sample 1 had 0M NaCl added while samples 2 to 7 had varying salt concentrations (0.8M-1.3M). These samples were prepared to determine the optimum salting-out concentration for loading.

Table 29: Desired and actual parameter values used in the preparation of the aqueous and salt extraction mediums (at the optimum salt concentration of 0.8M) and the mass of raw *Cicer arietinum* biomass added to the extraction medium in iteration 1

		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Desired NaCl molarity	**mol/l**	0	0.8	0.8	0.8	0.8	0.8	0.8
Volume of distilled water	**l**	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Desired mols of NaCl to add	**mol**	0	0.032	0.032	0.032	0.032	0.032	0.032
Desired mass of NaCl to add	**g**	0	1.87	1.87	1.87	1.87	1.87	1.87
Actual mass of NaCl added	**g**	0	1.87	1.862	1.861	1.875	1.863	1.862
Actual NaCl concentration	**mol/l**	0	0.8	0.797	0.796	0.802	0.797	0.797
Desired mass of biomass to add	**g**	2	2	2	2	2	2	2
Actual mass of biomass added	**g**	2.001	1.998	2.002	2	1.998	2.002	2.001

Table 30 shows the actual parameters that were used in aqueous loading (sample 1) and in salt loading (samples 2-7) of the extracted active components of *Cicer arietinum* in iteration 1. The latter involved the addition of salt to samples 3-7 to achieve the desired salt concentrations beyond the optimum salting-in concentration of 0.8M. The purpose of increasing the salt concentration further was to determine the optimum salting-out concentration for maximum adsorption. Table 31 shows the mass of ammonium sulphate added to the filtrate after filtration of the solution containing the loaded proteins. The resultant mass of precipitate from ammonium precipitation is shown in Table 32.

Table 30: Desired and actual parameter values used in the preparation of the aqueous and salt extraction mediums (with concentrations ranging from 0.8 to 1.3M) for loading of *Cicer arietinum* active components and the mass of silica added to the solutions in iteration 1

		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Desired NaCl molarity	**mol/l**	0	0.8	0.9	1	1.1	1.2	1.3
Volume of distilled water	**l**	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Desired mols of NaCl to add	**mol**	0	0.032	0.036	0.04	0.044	0.048	0.052
Desired mass of NaCl in solution	**g**	0	1.87	2.104	2.338	2.571	2.805	3.039
Desired mass of NaCl to add	**g**	0	0	0.242	0.477	0.696	0.942	1.177
Actual mass of NaCl added	**g**	0	0	0.244	0.476	0.698	0.944	1.179
Actual NaCl concentration	**mol/l**	0	0.800	0.901	1.000	1.101	1.201	1.301
Volume of solution	**ml**	38.8	39	38.7	39.1	38.8	39	39.2
Desired mass of silica to add	**g**	5.173	5.2	5.16	5.213	5.173	5.2	5.227
Actual mass of silica added	**g**	5.174	5.2	5.16	5.215	5.172	5.201	5.226

Table 31: The actual mass of ammonium sulphate added to the filtrate of samples 1 to 7 to achieve the desired ammonium saturation percentage following the loading of *Cicer arietinum* active components (iteration 1)

		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Saturation interval (%)		**Mass of ammonium sulphate added**						
0-20	**g**	4.56	4.561	4.563	4.558	4.559	4.562	4.56
20-40	**g**	4.9	4.909	4.922	4.916	4.92	4.895	4.901
40-60	**g**	5.316	5.278	5.297	5.286	5.274	5.277	5.283
60-80	**g**	5.727	5.735	5.717	5.726	5.736	5.726	5.722
80-100	**g**	6.28	6.286	6.286	6.287	6.287	6.28	6.28

Table 32: Mass of precipitate formed following loading of *Cicer arietinum* active components, ammonium precipitation, filtration and drying (iteration 1)

		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Mass of filter paper and watch glass	**g**	41.236	42	37.983	37.967	41.339	41.811	43.143
Mass of filter paper, watch glass and precipitate after drying	**g**	47.15	47.484	43.862	43.849	47.241	47.703	49.052
Mass of precipitate	**g**	5.914	5.484	5.879	5.882	5.902	5.892	5.909

A.3. Turbidity removal testing

In order to determine the volume of stock solution to add to 200ml of distilled water to achieve the desired turbidity of 200 NTU, different volumes of stock solution were added to a fixed volume of 200ml of distilled water and the turbidity was measured three times. The average and standard deviation of the three turbidity measurements were computed and is indicated in Table 33.

Table 33: Turbidity measurements of 200ml sample upon addition of varying volumes of stock solution

Volume of stock solution added (ml)	Turbidity (NTU)			
	Reading 1	Reading 2	Reading 3	Average ±standard deviation
0	3.03	3.02	3.04	3.03±0.01
1	35.4	35.1	34.5	35±0.37
2.2	48.4	49.2	49.7	49.1±0.54
3.6	56	54.2	53.3	54.5±1.12
5	85.7	91	85.5	87.4±2.55
6.6	108	104	106	106±1.63
8.6	127	125	133.5	128.5±3.63
10.8	143	146	147.5	145.5±1.87
13.4	199	201	200	200±0.82
16.4	252	248	247	249±2.16
20	306	299	301	302±2.94

The following sample of measurements (Tables 34-36) show the second iteration of turbidity testing of different doses of salt-extracted heterogeneous coagulant derived from *Cicer arietinum*.

Table 34: Second iteration of residual turbidity measurements (from 0 to 40 minutes) after application of different doses of salt extracted *Cicer arietinum* heterogeneous coagulant in a jar test

Dose(g/l)		0	2.5	5	10	15	20	25
Time(minutes)		**Residual turbidity (NTU)**						
0	**Reading1**	200	202	203	201.5	200	201	200.3
	Reading2	201	200.9	202.3	200.2	198.6	199.4	202
	Reading3	200.5	200	199	199	201	200.3	199
	Average	200.5	201	201.4	200.2	199.9	200.2	200.4
	σ	0.4	0.8	1.7	1.0	1.0	0.7	1.2
10	**Reading1**	198.1	177	173	169	178	179	188
	Reading2	197.8	177.9	172.9	165.2	176.5	174	185
	Reading3	197.8	177.6	170.9	166.4	178.3	181.4	187.8
	Average	197.9	177.5	172.3	166.9	177.6	178.1	186.9
	σ	0.1	0.4	0.9	1.6	0.8	3.1	1.4
20	**Reading1**	197.2	171.2	168.4	163.2	174.1	172.3	176.9
	Reading2	195	168.3	166	160.7	170.2	168.7	180.3
	Reading3	197.8	167.5	166.7	159.4	174.2	174.8	177.1
	Average	196.7	169.0	167.0	161.1	172.8	171.9	178.1
	σ	1.2	1.6	1.0	1.6	1.9	2.5	1.6
30	**Reading1**	196	144	137	120.8	123.4	126.5	124.9
	Reading2	197.4	148	128.4	123.9	124.6	131	127.3
	Reading3	196	149.2	132.1	115.7	124.7	123.2	126
	Average	196.5	147.1	132.5	120.1	124.2	126.9	126.1
	σ	0.7	2.2	3.5	3.4	0.6	3.2	1.0
40	**Reading1**	196.4	116.5	100.6	94.8	93.7	107.2	107.8
	Reading2	195.2	109.4	109.9	88.4	94.1	99.8	106.3
	Reading3	195.1	108.5	97.4	87.1	93.7	99.4	109.8
	Average	195.6	111.5	102.6	90.1	93.8	102.1	108
	σ	0.6	3.6	5.3	3.4	0.2	3.6	1.5

Table 35: Second iteration of residual turbidity measurements (from 50 to 60 minutes) after application of different doses of salt extracted *Cicer arietinum* heterogeneous coagulant in a jar test

Dose(g/l)		0	2.5	5	10	15	20	25
Time(minutes)		**Residual turbidity (NTU)**						
50	**Reading1**	194.2	104.8	87.4	64.8	68.2	70.9	77.2
	Reading2	192.8	108.2	88.1	61.3	66.9	65.8	75.2
	Reading3	194.5	104.8	84.4	63.7	67	69.6	74.9
	Average	193.8	105.9	86.6	63.3	67.4	68.8	75.8
	$\boldsymbol{\sigma}$	0.7	1.6	1.6	1.5	0.6	2.2	1.0
60	**Reading1**	194	64.2	53.9	41.8	45.6	53.1	56.1
	Reading2	192.6	58.7	56.3	40.2	47.2	50	52.1
	Reading3	190.3	60.4	55.7	42	46.9	54.3	57.3
	Average	192.3	61.1	55.3	41.3	46.6	52.5	55.2
	$\boldsymbol{\sigma}$	1.5	2.3	1.0	0.8	0.7	1.8	2.2

Table 36: Turbidity removal percentage for the second iteration of turbidity testing (over 60 minutes) using different doses of salt extracted *Cicer arietinum* heterogeneous coagulant

Dose(g/l)	0	2.5	5	10	15	20	25
Time(minutes)	**Average turbidity removal (%)**						
10	1.3	11.7	14.5	16.7	11.1	11	6.7
20	1.9	15.9	17.1	19.6	13.5	14.1	11.1
30	2	26.8	34.2	40	37.8	36.6	37.1
40	2.5	44.5	49	55	53.1	49	46.1
50	3.3	47.3	57	68.4	66.3	65.7	62.2
60	4.1	69.6	72.5	79.4	76.7	73.8	72.5

Appendix B: Sample calculations

B.1. Extraction of active components

The sample calculations shown in this section are for the first iteration of salt extraction of *Cicer arietinum* active components. In order to determine the optimum salting-in concentration for extraction, five samples (samples 2 to 6 in Table 25) of saline solutions with NaCl concentrations of 0.1M, 0.3M, 0.5M, 0.7M and 0.9M were prepared. To determine the mass of NaCl to add to 40ml of distilled water to achieve the desired molarities, the following calculations were used.

To achieve a NaCl concentration of 0.5M, the number of mols was determined by rearranging equation (B.1).

$$C = \frac{n}{V} \tag{B.1}$$

$$n_{NaCl} = C_{NaCl}.V \tag{B.2}$$

$n_{NaCl} = (0.5 mol/l)(0.04 l)$

$n_{NaCl} = 0.02\ mol$

The mass of NaCl to be added to 40ml distilled water to achieve a concentration of 0.5M NaCl was computed using the molar mass of NaCl (58.44g/mol) after rearranging equation (B.3),

$$n = \frac{m}{MM} \tag{B.3}$$

$$m_{NaCl} = n_{NaCl}.MM_{NaCl} \tag{B.4}$$

$m_{NaCl} = (0.02 mol)(58.44 g/mol)$

$m_{NaCl} = 1.169\ g$

Similar calculations were performed to determine the actual NaCl concentration based on the actual mass of NaCl added during experimentation.

The nomograph extracted from Spardaro, et al. (2003) was utilised to determine the mass of ammonium sulphate that should be added to each sample to achieve the desired change in ammonium sulphate saturation percentage.

The nomograph provided the mass of precipitate to add to 1000ml solution (summarised in Table 26), therefore at each saturation interval, the mass of ammonium sulphate to be added was scaled to determine the mass required for a 40ml solution. To saturate the solution from 40% to 60% ammonium sulphate, 132g of ammonium sulphate needs to be added to 1000ml of solution. Thus, the mass to be added to a 40ml solution was computed by scaling.

$$m_{(NH_4)_2SO_4} = 132g \times \left(\frac{40ml}{1000ml}\right) = 5.28g$$

The mass of precipitate formed following ammonium precipitation, filtration and drying was computed. For sample 4(in Table 28) the mass of precipitate was determined using equation (B.5).

$$m_{precipitate} = m_{precipitate+watch\ glass+filterpaper} - m_{watch\ glass+filterpaper} \quad \text{(B.5)}$$

$m_{precipitate} = 41.080g - 37.967g$

$m_{precipitate} = 3.113g$

B.2. Loading of active components

The sample calculations in this section are for the first iteration for the determination of the optimum salting-out concentration for improved loading of *Cicer arietinum* active components. The extraction of active components was firstly carried out at the optimum salting-in concentration; therefore, the calculation steps were similar to that presented in the extraction section B.1 and the calculation summary is shown in Table 29.

To determine the optimum salting-out concentration, more salt was added to samples 3 to 7(shown in Table 30) to achieve concentrations of 0.9M, 1M, 1.1M, 1.2M and 1.3M respectively. In order to calculate the mass of NaCl to add to each sample to achieve these concentrations, the required mass of NaCl to achieve the desired concentrations were firstly computed. To achieve a concentration of 0.9M, the mass of NaCl was computed using equation (B.2).

$n_{NaCl} = C_{NaCl}.V$

$n_{NaCl} = (0.9mol/l)(0.04l)$

$n_{NaCl} = 0.036\ mol$

The mass of NaCl added to 40ml distilled water to achieve 0.9M was calculated using equation (B.3).

$m_{NaCl} = n_{NaCl}.MM_{NaCl}$

$m_{NaCl} = (0.036mol)(58.44g/mol)$

$m_{NaCl} = 2.104\ g$

The mass of NaCl already added for extraction (1.862g) was then subtracted from this mass, the mass of NaCl added to achieve the concentration of 0.9M was computed as follows,

$m_{NaCl} = 2.104g - 1.862g$

$m_{NaCl} = 0.242g$ additional NaCl

Similarly, the actual NaCl concentration of each sample, after the salt addition, was finally computed.

In order to determine the mass of silica to be added to solution, a ratio of silica mass to protein-rich solution volume of 1.2g of silica per 9ml of solution was applied (Jerri, et al., 2012).Thus, for the volume of sample 3(38.7ml), the required silica addition was computed as follows,

$$m_{SiO_2} = 1.2g \times \left(\frac{38.7ml}{9}\right)$$

$$m_{SiO_2} = 5.16g$$

Table 26 was utilised to determine the mass of ammonium sulphate required for each addition to achieve 100% saturation. The mass of precipitate was computed in a similar manner to that showcased in the extraction section B.1.

B.3. Turbidity removal testing

Three measurements of turbidity were made after the addition of a fixed volume of stock solution to 200ml distilled water to determine the relationship between dose of stock solution and turbidity. The average turbidity was computed as follows for a volume addition of 1ml stock solution (from Table 33).

$$Turbidity_{av} = \frac{Reading\ 1 + Reading\ 2 + Reading\ 3}{3} \tag{B.6}$$

$$Turbidity_{av} = \frac{35.4NTU + 35.1NTU + 34.5NTU}{3}$$

$$Turbidity_{av} = 35\ NTU$$

The standard deviation (σ) was computed,

$$\sigma = \sqrt{\frac{\sum(x - \bar{x})^2}{n}} \tag{B.7}$$

$$\sigma = \sqrt{\frac{(35.4-35)^2+(35.1-35)^2+(34.5-35)^2}{3}}$$

$$\sigma = 0.374$$

Linear regression was utilised to determine the relationship between stock solution dose and average turbidity. Volume of stock solution added was denoted ‘x’ since it was the independent variable and average turbidity was denoted ‘y’, as it was the dependent variable.

The mean of the volume of stock solution added (x) was computed.

$$\bar{x} = \frac{\sum_i x_i}{n} \quad \text{(B.8)}$$

Where, x_i was the i^{th} volume measurement in the data set and 'n' was the total number of samples (volume measurements) in the data set.

$$\bar{x} = \frac{0 + 1 + \ldots + 20}{11}$$

$$\bar{x} = 7.955$$

Similarly, the mean of the average turbidity measured (y) was determined.

$$\bar{y} = \frac{\sum_i y_i}{n} \quad \text{(B.9)}$$

Where, y_i was the i^{th} average turbidity measurement in the data set and 'n' was the total number of samples (average turbidity measurements) in the data set.

$$\bar{y} = \frac{3.03 + 35 + \ldots + 302}{11}$$

$$\bar{y} = 123.63$$

The sum of squares of x's (S_{xx}) was then computed.

$$S_{xx} = \sum_i (x_i{}^2) - \frac{(\sum_i x_i)^2}{n} \quad \text{(B.10)}$$

$$S_{xx} = (0^2 + 1^2 + \ldots + 20^2) - \frac{(0 + 1 + \ldots + 20)^2}{11}$$

$$S_{xx} = 426.78$$

Similarly, the sum of squares of y's (S_{yy}) was determined.

$$S_{yy} = \sum_i (y_i{}^2) - \frac{(\sum_i y_i)^2}{n} \quad \text{(B.11)}$$

$$S_{yy} = (3.03^2 + 35^2 + \ldots + 302^2) - \frac{(3.03 + 35 + \ldots + 302)^2}{11}$$

$$S_{yy} = 88229.46$$

The sum of products of x and y (S_{xy}) was thereafter established.

$$S_{xy} = \sum_i x_i y_i - \frac{(\sum_i x_i)(\sum_i y_i)}{n} \quad \text{(B.12)}$$

$$S_{xy} = (0)(3.03) + (1)(35) + \ldots (20)(302) - \frac{(0 + 1 + \ldots + 20)(3.03 + 35 + \ldots + 302)}{11}$$

$$S_{xy} = 6112.245$$

The gradient (b) and y-intercept (a) of the least squares regression line was then determined,

$$b = \frac{S_{XY}}{S_{XX}} \tag{B.13}$$

$$b = \frac{6112.245}{426.78}$$

$$b = 14.322$$

$$a = \bar{y} - b\bar{x} \tag{B.14}$$

$$a = 123.63 - (14.322)(7.955)$$

$$a = 9.6999$$

Thus, the equation of the fitted regression line is

$$y = 14.322 - 9.6999x$$

To assess the goodness of fit of the regressed line, the coefficient of determination (R^2) was computed.

$$R^2 = \frac{b^2 S_{XX}}{S_{YY}} \tag{B.15}$$

$$R^2 = \frac{(14.322)^2\,(426.78)}{(88229.46)}$$

$$R^2 = 0.9922$$

In the determination of the optimum dose of heterogeneous coagulant, three residual turbidity measurements were recorded every 10 minutes for 60 minutes (shown in Tables 34 and 35). These values were averaged, and the average turbidity removal percentage was determined.

At a salt extracted *Cicer arietinum* coagulant dose of 2.5g/l, the average residual turbidity after 10 minutes was computed as follows (using equation (B.6)),

$$Turbidity_{av} = \frac{Reading\ 1 + Reading\ 2 + Reading\ 3}{3}$$

$$Turbidity_{av} = \frac{177NTU + 177.9NTU + 177.6NTU}{3}$$

$$Turbidity_{av} = 177.5\ NTU$$

The standard deviation (σ) was thereafter calculated using equation (B.7).

$$\sigma = \sqrt{\frac{\sum(x-\bar{x})^2}{n}}$$

$$\sigma = \sqrt{\frac{(177-177.5)^2+(177.9-177.5)^2+(177.6-177.5)^2}{3}}$$

$$\sigma = 0.37$$

The average turbidity removal can finally be determined using equation (2.1) (shown in Table 36).

$$\%\ Turbidity\ Removal = \frac{T_i - T_f}{T_i} \times 100$$

Where, T_i is the initial turbidity measurement of the sample and T_f is the measured turbidity after application of the coagulant.

$$\%\ Turbidity\ Removal = \frac{201NTU - 177.5NTU}{201NTU} \times 100$$

$$\%\ Turbidity\ Removal = 11.7\%$$

Appendix C: Experimental diagrams

C.1. Experimental materials

Figure 49: *Vigna mungo* seeds used in experimentation

Figure 50: *Cicer arietinum* seeds used in experimentation

Figure 51: *Glycine max* seeds used in experimentation

Figure 52: *Vigna unguiculata* seeds used in experimentation

C.2. Experimental equipment

Figure 53: Russell Hobbs domestic coffee grinder

Figure 54: Kern & Sohn digital scale

Figure 55: Labcon shaking incubator oven

Figure 56: Lasec reciprocating shaker

Figure 57: Frontier multi centrifuge

Figure 58: Lovibond infrared turbidimeter

C.2. Turbidity testing

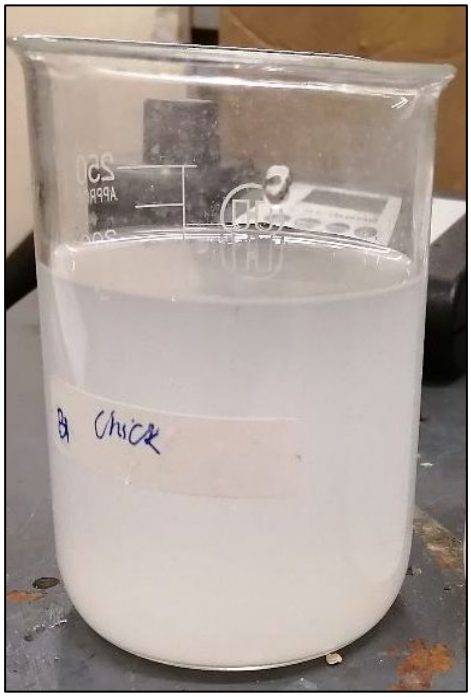

Figure 59: Optimised aqueously extracted heterogeneous coagulant (*Vigna mungo*) in turbid water after 0mins of sedimentation

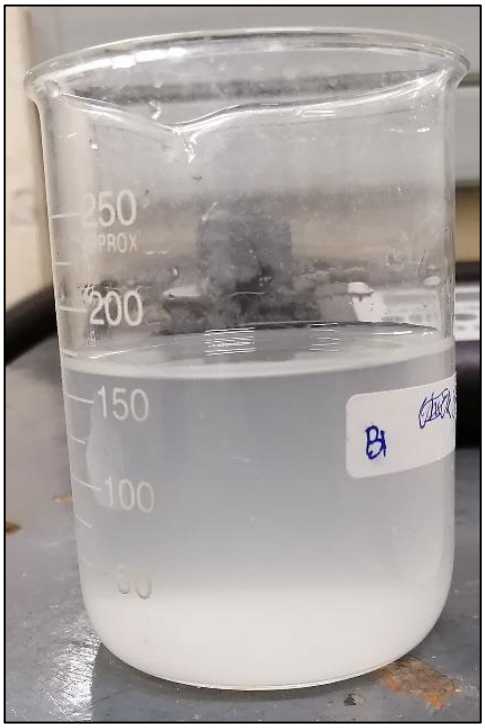

Figure 60: Optimised aqueously extracted heterogeneous coagulant (*Vigna mungo*) in turbid water after 60 minutes of sedimentation

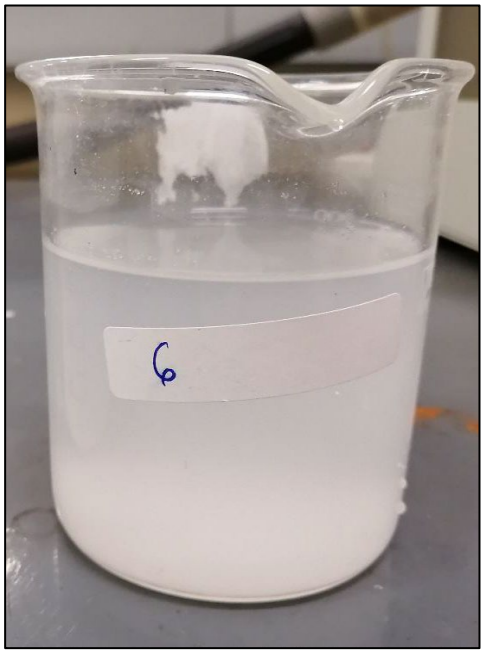

Figure 61: Optimised salt- extracted heterogeneous coagulant (*Vigna mungo*) in turbid water after 0mins of sedimentation

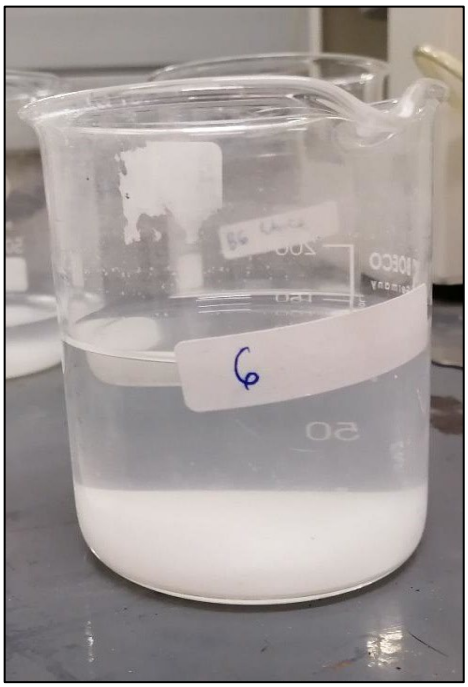

Figure 62: Optimised salt-extracted heterogeneous coagulant (*Vigna mungo*) in turbid water after 60minutes of sedimentation

Appendix D: Safety precautions

The following safety requirements must be met when working in the laboratory:

- A lab coat, safety shoes and safety goggles must be worn at all times
- Ensure all connections are correct by consulting the lab technician in charge before turning on any equipment.
- Electrical apparatus and connections must be kept away from moisture.
- Handle all equipment with care and if there are any breakages, inform the lab technician immediately.
- Ensure that all equipment is properly shut down before leaving the laboratory.