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# Natural organic matter (NOM) and turbidity removal by plant-based coagulants: a review

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**Abstract.**

NOM deteriorates water quality by forming taste, clarification, colour, and odour problems. It also increases coagulant and chlorine consumption which can initiate disinfection by-products harmful to human health. The coagulation-flocculation (CF) technique is an established method commonly employed to remove NOM in water treatment. Plant-based coagulant products (PCPs) derived from plants like the *Moringa oleifera* (MO) *Strychnos potatorum Linn* and *Opuntia ficus indica,* have been studied and proposed as sustainable alternatives to chemical coagulant, like, aluminium sulphate due to their abundant availability, low cost, low sludge volume and disposal cost, and biodegradability. This review paper provides an overview of the most widely studied plant-based coagulants and discusses their NOM and turbidity removal. It investigates recent analytical tools applied in their characterisation and floc morphological studies. The paper also investigates the effects of operating parameters such as coagulant dose, temperature, and pH, on NOM and turbidity removal. It also reviews up-to-date PCPs biophysical properties and CF mechanism and examines the efficiency of their extraction methods in reducing NOM. Finally, it discusses and suggests ways to overcome commercialisation draw-back caused by nutrient addition.

Keywords: coagulation-flocculation; coagulant; drinking water; organic matter; compounds extraction and purification

**Contents**

**List of abbreviations**

2,3-epoxypropyl trimethyl ammonium chloride (GTA); Absorbance slope index (ASI); Apparent molecular weight (AMW); Cationic starch-based flocculant (St-CTA); Change in UV/Vis absorbance (ΔA); Chemical Coagulant Products (CCPs); Circular dichroisms (CD); Coagulation-flocculation (CF); Computational fluid dynamics (CFD); Disinfectant by-products (DBPs); Dissolved organic carbon (DOC); Dissolved organic matter (DOM); Electron microscopy (EM); Excitation-emission matrix (EEM); Fluorescence regional integration (FRI); Fourier-transform infrared spectroscopy (FT-IR); Gas chromatography–mass spectrometry (GC-MS); Haloacetic acid (HAA); Haloacetic acid formation potential (HAAFP); High-performance size exclusion chromatography (HPSEC); Household water treatment and storage (HWTS); Hydrophilic acid (HPI); Hydrophobic acid (HPO); Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP AES); Isoelectric point (IEP); Laser in-situ scattering and transmissiometery (LISST); Liquid chromatography–mass spectrometry (LC–MS); Mass analysis (MS); Maximum contaminant limit (MCL); Molecular weight (mW);

Natural organic matter (NOM); N-nitrosodiethylamine (NDEA); N-nitrosodimethylamine (NDMA); N-nitrosodiphenylamine (NDPhA); N-nitrosodipropylamine (NDPA); N-nitrosopyrrolidine (NPYR); Nuclear Magnetic Resonance (NMR): Parallel factor analysis (PARAFAC); Plant Coagulant Products (PCPs); Polyacrylamide (PAM); Polyaluminum chloride (PAC); Potassium chloride (KCl); Principle component analysis (PCA); Size exclusion chromatography (SEC); Small-angle laser light scattering (SALLS); Sodium Chloride (NaCl); Specific ultraviolet absorbance (SUVA); Total dissolved solids (TDS); Total organic carbon (TOC); Total suspended solids (TSS); Trihalomethanes (THM); Trihalomethanes formation potential (THMFP); Ultrafiltration (UF); Ultra-small-angle neutron scattering (USANS); Ultraviolet-visible spectroscopy (UV/Vis); United States Environmental Protection Agency (US EPA); World Health Organisation (WHO)

## 1 Introduction

Untreated water contains a complex mix of both natural and synthetic organic and inorganic compounds. Naturally occurring organic matter (NOM) is autogenic and originates from biological, geological, and hydrological activities such as pathogenic organism, detritus litter and photosynthetic eukaryotic organisms (Matilainen *et al.*, 2002, Fu *et al.*, 2017). At the same time, compounds of synthetic origin are anthropogenic, resulting from industrial activities. NOM present in untreated water may be suspended or dissolved and can affect water quality by impacting on its appearance, colour, taste and odour, thereby making it undesirable. It can also be a medium for the transportation of other harmful chemicals and particles, and a precursor to the formation of harmful compounds during water disinfection (Matilainen *et al.*, 2011). NOM particles are of submicron sizes and are composed of smaller, loosely bonded particles, irregularly shaped and porous (Jarvis *et al.*, 2005). They predominantly carry negative surface charges due to their functional groups' ionisation reaction and surface adsorption of charged species like polymers (Jarvis *et al.*, 2005). The negative charges are

responsible for their stability, which makes floc formation and settling out very difficult. In water treatment, NOM removal occurs once their composition and quantity have been examined (Matilainen *et al.*, 2011). Commonly used NOM treatment processes are coagulation and flocculation (CF) followed by sedimentation and filtration, activated carbon filtration, membrane filtration, advanced oxidation processes, and ion exchange resin.

In the CF process, coagulants such as metallic salts, e.g., aluminium and ferric, are used to destabilise stable NOM solutions into micro flocs, which later settle-out by gravity sedimentation. Several studies have reported improved flocs properties and NOM removal using these metallic salts and their derivatives (Sillanpää *et al.*, 2018). Notwithstanding their wide usage, they still have some limitations as seen in their acquisition cost, large sludge volume and ecotoxicological concerns both in treated water and sludge (Oladoja, 2015). Chemical coagulant products (CCPs) such as aluminium salts have been linked to health problems like Alzheimer disease (Exley, 2017), and their performance largely depends on their hydrolysing pH (Sillanpää *et al.*, 2018). A slight alteration of their pH may favour or reduce charged species that can influence colloid agglomeration rate (Sillanpää *et al.*, 2018). These limitations have led to an exponential increase in nature-based water treatment research that aims to provide alternative solutions that overcome the limitations of the CCPs.

Nature-based coagulants date far back to 77AD, as noted in Roman archives (Dorea, 2006), and have been used for water purification since then. Other early documentation on plant coagulation products (PCPs) use for water treatment includes ancient Sanskrit writings and manuscripts (Jahn, 1981) which revealed treatment efficacy of *Strychnos potatorium*, *Phyllantus embrica* and *Luffa cylindrical*. These green and eco-friendly coagulants come from plant roots,barks, stems, leaves, seedpods and flowers (Choy *et al.*, 2015); animal bones, shells, the exoskeleton of shellfish and scales (Choy *et al.*, 2015), and natural minerals found in soils (ALI *et al.*, 2004). Of all the nature-based coagulants, the PCPs are the most accessible, low

132 cost, degradable, environmentally safe and carbon-neutral, regenerative in nature, widely
133 distributed and easy to prepare and handle (Choy *et al.*, 2015). Recently, PCPs such as *Moringa*
134 *oleifera*, *Opuntia ficus indica* and different Hibiscus species have been used to remove high
135 turbidity and organic matter from water (Pramanik *et al.*, 2015). These PCPs have great
136 prospects as confirmed by their successful use in household water treatment and storage
137 (HWTS) and small community water supply schemes (Jahn, 1981, Marobhe *et al.*, 2007).

138 Several reviews of PCP application in water treatment reported on their extraction and
139 compounds purification e.g. (Kansal and Kumari, 2014, Oladoja, 2015), general coagulation
140 effectiveness and mechanism e.g. (Bolto and Gregory, 2007, Choy *et al.*, 2015, Oladoja, 2015,
141 Saleem and Bachmann, 2019), cost and marketing constraints e.g. (Yin, 2010, Kansal and
142 Kumari, 2014, Saleem and Bachmann, 2019) and toxicity e.g. (Bolto and Gregory, 2007, Yin,
143 2010). From the reviews and recent publications, the main challenges hindering PCP use are
144 nutrient addition to the treated water, resulting in odour and colour problems, reduced treatment
145 efficiency, and increased potential to form disinfection by-products (DBPs) in chlorinated
146 systems. Most of the compounds present in the PCPs, such as carbohydrate, proteins and
147 carboxylic acids, are potential precursors to the formation of DBPs such as trihalomethanes
148 (THM), haloacetic acids (HAA), halonitromethanes, haloketones (Richardson and Ternes,
149 2018), but detailed studies of the role of PCPs in introducing these precursors when used for
150 water treatment are lacking.

151 Furthermore, only a small percentage of PCP studies have characterized the flocs generated,
152 and examined nutrients contribution by the PCPs, thus resulting in a gap in our understanding
153 of their use in CF treatment. Also, there is a dearth of a comprehensive overview addressing
154 critical appraisal of PCP's biophysical properties, their NOM removal performance under
155 different operating conditions such as pH, temperature, and their purification procedures. In
156 the light of the above, the aim of the present review highlights the knowledge gaps in these

areas. Emerging recommendations from this review are anticipated to help in optimising their use for medium- and industry-scale applications. This review also adds to the existing knowledge base to and motivate water managers, decision-makers, and researchers to undertake further work on them.

## 2 Overview of the Principle of coagulation-flocculation process and factors affecting NOM removal in PCPs coagulated water

The CF process is a low-cost technique for destabilising very small particles, so they form larger flocs that are easily removed through sedimentation or filtration. The fine particles being coagulated cause turbidity, colour, and odour, and are potential precursors to DBP formation in chlorinated water. The CF process (Fig. 1), starts by adding a coagulant to untreated water to reduce the natural repulsion between particles. Then the water is gently mixed to encourage particles to clump into flocs. NOM particles are predominantly negatively charged (Bouaouine *et al.*, 2018). The coagulant must be capable of neutralising the particle charges (otherwise known as particle destabilisation) or have the ability to trap, bridge or encapsulate them in a process termed adsorption and bridging. Ionic charge on coagulants can be cationic, ionic, poly-ionic, or non-ionic, making them behave either as coagulants, flocculants, or both. The differences between these PCP types are discussed in Section 3.2.

After particle destabilization, coagulants form micro and macro flocs through the charge neutralization process (Bolto and Gregory, 2007). However, poor results from the coagulation process may require adding a flocculating agent often known for improving water quality and floc characteristics. Flocculant aids help clump the small, destabilised particles into more extensive and denser aggregates to enable quicker settling rates. Most PCPs act both as coagulants and flocculants, making them a promising additive for the CF water treatment process.

The CF mechanism often involves more than one of these mechanisms depending on the water chemistry and type of PCP used. With the wide variability of the organic compound types, molecular weights and the electrostatic charge on both NOM and the PCPs, the overall system performance in NOM removal will differ between water sources and different PCP used. The following section gives an insightful discuss into factors responsible for NOM removal in PCPs coagulated water.

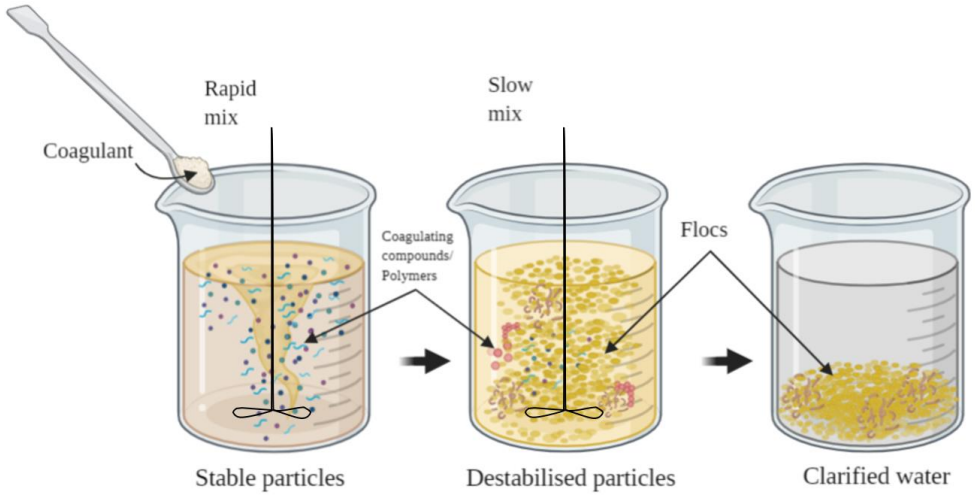



**Fig. 1 Flocs formation process by PCPs**

## 2.1 Role of the water chemistry

The effective elimination of turbidity and NOM removal in treated water depends on both the properties of the PCPs (solubility, dosage, surface charge and basicity) and the untreated water quality (pH, alkalinity, co-existing ions, solids content, and temperature) (Fig. 2). It may therefore sometimes be necessary to adjust one or several of these parameters in a pre-treatment step to achieve a desirable performance (Nath *et al.*, 2020). The removal of NOM depends on the water source and the nature of the suspended, colloidal, and dissolved organic constituents. NOM composition and concentration generally controls the performance of other treatment variables. High organic matter concentration, with their corresponding high surface charges, can dominate the CF process and improve the neutralization of the colloidal particles resulting

to a high turbidity and NOM removal (Kim *et al.*, 2001, Choy *et al.*, 2015). The presence of multi-charged ions such as bivalent ions of calcium or magnesium could assist the CF process (Okuda *et al.*, 2001b, Oladoja, 2015). Several researchers have demonstrated how the addition of these ions can help to reduce the organic matter concentration and residual turbidity (Tripathi *et al.*, 1976, Okuda *et al.*, 2001b).

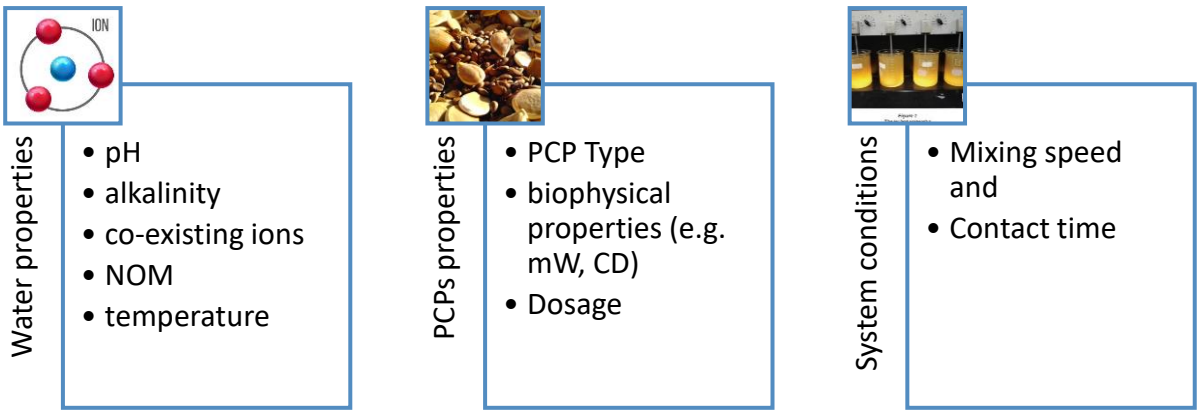



**Fig. 2 Factors affecting NOM removal in PCPs coagulated water**

Alkalinity level in a water, also referred to as its buffering potential, shows its ability to neutralize acid contaminants (Priyatharishini and Mokhtar, 2020). During the CF process, alkalinity and pH are closely related, with high alkalinity waters mostly having high pH causing changes in chemistry of the coagulant in terms of PCPs solubility and NOM surface charges. High alkalinity level in water may require higher PCP dosages to decrease the pH to a favourable value for effective coagulation (Tseng *et al.*, 2000). Alkalinity is not harmful, however its high concentration in water can increase dissolved solids and hardness level (McNeely *et al.*, 1979). More so, a decrease in pH, alkalinity, and increase in ionic strength are closely related and could cause an imbalance in the water chemistry thus affecting taste and posing the risk of corrosion (Shah *et al.*, 2012, Khan *et al.*, 2013, Radfard *et al.*, 2018). Unlike chemical coagulants, such as the aluminium sulphate, that affect water pH and alkalinity, most PCPs have minimal effect on these measured parameters (Table 1), and no known record of pre-alkalinisation exists in PCPs water treatment to the authors knowledge.

Several researchers have reported insignificant changes in alkalinity of water treated with *Moringa oleifera* seeds (Ndabigengesere and Subba Narasiah, 1998), *Hibiscus rosa sinensis* leaf extract (HLE) (Nidheesh *et al.*, 2017) and Jackfruit peel (*Artocarpus heterophyllus*) (Priyatharishini and Mokhtar, 2020). On the other hand, *Cactus opuntia* showed a decrease in the coagulant performance after increasing the alkalinity (Diaz *et al.*, 1999, Zhang *et al.*, 2006). So, changes to alkalinity seem to differ by PCP types, which implies that coagulant selection is vital for the overall success of the treatment process. More details on the PCPs biophysical properties and the PCP selection process are discussed in Section 3.2.

Table 1 PCPs impact on water alkalinity

| Coagulant | Test sample | Initial conditions | Final conditions | Reference |
|---|---|---|---|---|
| *M.oleifera* seeds. | Kaolin solution 1mL/L dosage; 105 NTU; pH 7.6 | Alkalinity: 53mg/ L; Conductivity: 154mmho/cm | Alkalinity: 53mg/ L; Conductivity: 154mmho/cm | (Ndabigengesere and Subba Narasiah, 1998) |
| *Trigonella foenum graecum* seeds | Pond water 10mL/L dosage; 228 ± 34; pH 7.12 | Alkalinity: 126 ± 16mg/ L | Alkalinity: 74 ± 9mg/ L | (Ramamurthy *et al.*, 2012) |
| *M.oleifera* seeds. | Ground water 150mg/L dosage; 12.4 NTU; pH 8.0 ± 0.05 | Alkalinity: 130 ± 0.1mg/L; Hardness: 190 ± 0.57mg $CaCO_3$/L | pH 7.2 ± 0.5; Alkalinity: 100 ± 0.28 mg/L; Hardness: 100 ± 0.57mg $CaCO_3$/L | (Mangale Sapana *et al.*, 2012) |
| *M.oleifera* seeds. | 4.5 mL/L 150mg/L dosage; 20.5 NTU; pH 6.8 | Alkalinity: 32mg/L; Hardness: 17.1 | pH 6.9; Alkalinity: 30; Hardness: unchanged | (Egbuikwem and Sangodoyin, 2013) |
| *Parkinsonia aculeate* seeds | Dam water 6mg/L dosage; 810 NTU; pH 7.4 | Alkalinity: 74mg/ L | Alkalinity: 52.5mg/ L | (Marobhe *et al.*, 2007) |

PCPs effectiveness in removing NOM during CF treatment is affected by high or low pH. In optimum conditions, the coagulation pH influences the charge of the NOM functional group,

the surface charge of colloids, and the charge of the dissolved phase solubility, thus affecting the stability of the suspension (Yan *et al.*, 2008, Yang *et al.*, 2009). For a specific raw water and PCP type, the optimum pH affects the hydrolysate from both NOM and the coagulating compounds present in the PCPs (Sun *et al.*, 2016, Sun *et al.*, 2019). At low pH, the negatively charged humus colloid is easily removed, whereas at high pH, the humus hydrophilicity is enhanced making its removal difficult (Sun *et al.*, 2019). Since PCPs have unique CF effect, optimum pH should be established based on PCP type, raw water type and treatment variables (Fig. 2), through experimental techniques such as the Jar test.

Coagulation compounds have a different rate of solubility depending on the pH of the solution. High pH can result in high molar mass flocs, whereas lower solution pH leads to the formation of polymers of medium or low molar mass (Yan *et al.*, 2008). At low pH, e.g. pH 4.0, some PCPs such as banana pith cause protonation of functional groups such as the amino and carboxyl group, leading to higher cationic colloidal electrolytes in solution (Kakoi *et al.*, 2016), favouring destabilisation of the suspended particles. Conversely, higher pH, e.g. pH 9.5, makes PCPs such as *Phaseolus vulgaris*, become anionic (Šćiban *et al.*, 2009) and the coagulation mechanism forms a net-like structure due to attraction of bivalent cations in water, causing enmeshment and then removal of particles by sweep flocculation.

Different PCPs have a unique solution pH at which their optimum NOM and turbidity removal occurs. At room temperature of 18$^{o}$C, Okra salt extract has the best turbidity removal (99%) at pH 4.0 (Jones and Bridgeman, 2016). Water and saline (1M NaCl) products of Common Oak (*Quercus robur*), *Aesculus hyppocastanum* (Horse chestnut), *Quercus cerris* (Turkey oak), *Quercus rubra* (Northern red oak) and *Castanea sativa* (European chestnut), had their optimum pH between 7.0 and 10.0 (Šćiban *et al.*, 2009). For *Opuntia focus-indica* (nopal), the optimum NOM removal was at pH 10.0 (Zhang *et al.*, 2006). A few works of literature prove that

understanding the optimum solution pH can result in substantial improvement of NOM removal. So, PCPs pH can be controlled or adjusted if necessary.

Besides the pH and alkalinity, the presence of concentrations of ions such as chloride, bicarbonate, and sulphate in the feed water, can influence PCPs performance. These electrolytes have shown to reduce the percentage turbidity removal in cactus treated water from 98% to 66% (Choudhary *et al.*, 2019). High concentration of these electrolytes causes higher electrostatic repulsions, influencing both the colloidal particle-particle interaction as well as coagulant-particle interactions. On the other hand, the presence of multi-charged ions such as bivalent ions of $Ca^{+2}$, $Ba^{2+}$ and $Mg^{+2}$ could assist the CF process and help to reduce the organic matter concentration and residual turbidity by enmeshing particles in a net-like structure (**Fig. 3**) and removing by a sweep coagulation action, which supports the charge neutralization process (Tripathi *et al.*, 1976, Okuda *et al.*, 2001b, Okuda *et al.*, 2001a, Šćiban *et al.*, 2009, Šćiban *et al.*, 2021). The presence of these divalent ions has a synergistic effect on the CF process by enhancing formation of complexes (Arunkumar *et al.*, 2018, Choudhary *et al.*, 2019, Okoro *et al.*, 2021) as subsequently illustrated in Fig. 4. Contrary to the above findings, some researchers working on MO and HLE noted that concentration of both anion and cation has no significant effect on the PCPs performance (Nidheesh *et al.*, 2017). So, the influence of co-existing ions in the feed water on the coagulation efficiency might be a unique attribute and should be established before choosing any treatment program.

Another scarcely discussed variable, water salinity level, potentially can affect the solubility of PCPs proteins and polysaccharides, thereby affecting the extent of particle bridging (Oladoja *et al.*, 2017, Choudhary *et al.*, 2019). On the other hand, it can improve water treatment performance by reducing the electrostatic repulsion between colloidal particles and coagulating compounds, improving bridging interactions that enhance particle sedimentation (Megersa *et al.*, 2019). High salinity level decreases the electric double layer of suspended particles due to

electrolyte compression, resulting in increased collision frequency due to reduced particles repulsion (Oladoja *et al.*, 2017, Megersa *et al.*, 2019).

## 2.2 Influence of dosage, and temperature

Coagulant dosage affects the efficiency of the treatment process. Doses which are too high or too low impacts sludge formation (Ibrahim and Aziz, 2014), and causes reduction of NOM reactive surface, thus leading to low adsorption by the coagulant (Camacho *et al.*, 2017, Yan *et al.*, 2008). Certain ions present in the PCPs at certain doses can induce the required ionic strength needed for double-layer compression (Diaz *et al.*, 1999, Miller *et al.*, 2008) resulting in coagulation. This concept is still largely unclear and requires more research effort. Overdosing also causes leaching of nutrients to the coagulated water (Okoro *et al.*, 2021). So, determining the right amount of coagulants dosage is essential for best NOM removal efficiency.

Heating the PCPs can denature or improve the structure of the coagulating compounds which can influence NOM removal. Some heated PCPs, such as autoclaved corn and rice starch, experienced an increase in turbidity removal, exceeding those of crude wheat and corn starch (Choy *et al.*, 2016). This research agrees with the earlier findings of Bodlund *et al.* (2014), who recorded a twofold improvement in coagulation performance of Mustard seeds after heat treatment. Other reports evidencing improved turbidity removal after heat treatment includes Okra salt products (Jones and Bridgeman, 2016), small, large and yellow water extracts of Mustard seeds (Bodlund *et al.*, 2014), and .

Aside from temperature-induced changes caused during the PCP purification, the source water temperature can also influence NOM removal performance. Temperature acts as the driving force for the CF treatment process and affects the PCP's ability to aggregate particles. Temperature also influences the physical properties of the PCPs including their solubility,

mobility, viscosity, density, collision, and settling velocity of the flocs. High temperature speeds up the reaction rate, whereas low temperature stabilises the colloidal surfaces causing lower rate of hydrolysis (Scholz and Scholz, 2016, Watanabe, 2017). On the contrary, low temperature generally reduces NOM and turbidity removal efficiency. While comparing temperatures of 10$^{o}$C, 20$^{o}$C and 35$^{o}$C using 50 mg/L of *Opuntia indica* flour, Zhang *et al.* (2006) reported that water temperature of 10$^{o}$C (residual turbidity: 7.8 NTU) produced the worst result compared to 20$^{o}$C (residual turbidity: 5.6 NTU) and 35$^{o}$C (residual turbidity: 5 NTU), and solids removal seemed to increase with increasing temperature. Their observation could be attributed to the particles settling behaviour. During the CF process, most colloidal particles settle within the range of validity of Stokes' law, with their settling velocity inversely varying as the viscosity of the water, resulting in longer settling period when the water viscosity is high, i.e., during winter, than in the summer months (Camp *et al.*, 1940). For instance, in summer, water temperature of say 20°C may enable particles to settle up to 50% faster than in winter when water temperature is perhaps 10°C.

The result is independently supported by Hanson and Cleasby (1990), who on increasing MaganaflocLT-22 polymer dose from 0.05 to 0.2 mg/L under cold temperature of 1.0$^{o}$C, notice that solids removal remained unchanged. A plausible conclusion from this was that the main mechanism responsible for coagulation under cold temperatures was charge neutralization and that the sweeping up action was possibly affected by the increased water viscosity (Hanson and Cleasby, 1990). This dependency of turbidity and NOM removal on temperature suggests that a higher number of water hydrogen bonds/ polymer are broken by increasing the temperature, thus strengthening the hydrophobic interactions between the suspended particles-polymers (Ng *et al.*, 2017, Vajihinejad *et al.*, 2019). So, the temperature can both affect PCP structure before and during the CF process. Due to limited literature on temperature effect during PCPs use,

327 further research work will help confirm if the PCP's coagulating compounds and reaction
328 kinetics changes with temperature.

329 **2.3 Influence of storage duration**

330 The major challenge in extended storage of PCPs is the denaturing of coagulation compounds,
331 reducing their NOM removal ability. Coagulation compounds such as protein are sensitive to
332 temperature and can quickly degrade or become completely inactive depending on their type
333 and storage temperature. There is currently no fixed storage duration for the PCPs because of
334 their coagulating compounds' unique properties. Some of these PCPs remain efficient only for
335 24 hours, while others can last up to 5 days (Warrier *et al.,* 2014). PCPs, especially those in
336 solution form, are stored in cold conditions (especially purified products) to prevent
337 denaturation as seen in a stock solution of *Strychnos potatorum* (Warrier *et al.,* 2014). The
338 solutions could not last beyond 5 days due to the degradation of proteins. Contrarily, some
339 PCPs stored at room temperature, as might be expected in most HWTS, had sustained NOM
340 removal efficiency even after 10 days (Katayon *et al.,* 2006), which could be beneficial to those
341 without no access to cold storage. Extended storage can also degenerate coagulated water
342 quality by causing resuspension of weak flocs (Katayon *et al.,* 2006). It can also support
343 pathogens' growth due to the high nutrient content of coagulated water, leading to increased
344 DOC of water - a recognised precursor to the formation of disinfection by-products.

345 The majority of water treatment studies using PCPs evaluate treatment performance using
346 water quality indices such as suspended and dissolved solids (TSS/TDS) and turbidity level,
347 with some considering colour. These visual parameters only partially estimate NOM
348 concentration without showing detailed properties and character of the NOM. The following
349 sections present standard tools for characterising NOM in PCP coagulation water to assess their

350 efficiencies and adequacy for use during drinking water treatment. Table 2 gives an overview
351 of these tools and their limitation.

352 **Table 2 Commonly used method for characterising natural organic matter (NOM) in PCP**
353 **coagulated water. Adapted from (Matilainen *et al.*, 2011)**

| Method with references | Detected features | Performance | Reference |
|---|---|---|---|
| *Bulk NOM indicator measurement* | | | |
| TOC | Total organic carbon content in the coagulated water | Low cost and analytical skill required.<br>Only information on NOM quantity provided | (Altaher *et al.*, 2016) |
| DOC | Dissolved organic carbon content in the coagulated water after filtering water through 0.45µm filter | Same as TOC | (Anastasakis *et al.*, 2009, Pramanik *et al.*, 2015, Jones and Bridgeman, 2016, Okoro *et al.*, 2021) |
| SUVA | SUVA > 4 indicates aromatic, hydrophobic, high chlorine demand compounds, while SUVA < 3 shows compounds that are mainly hydrophilic with low chlorine consumption. | It is quickly determined using information UV and DOC. | (Baptista *et al.*, 2015, Baptista *et al.*, 2017, Okoro *et al.*, 2021) |
| Spectroscopic techniques | | | |
| Fluorescence | Compounds present in the water are excited on exposure to radiation. Several peaks represent different functional groups present in PCPs | High analytical speed and sensitivity<br>Result affected by water chemistry such as pH. Require advance analysis (FRI, PARAFAC) to quantity NOM | (Coble, 1996, Jones and Bridgeman, 2019) |
| UV/Vis | Quantifies all UV-light-absorbing compounds present in the water. | Simple and easy to use<br>May be affected by pH, high turbidity, and solvent polarity | (Korshin *et al.*, 2009, Priya *et al.*, 2017) |

| | | | |
|---|---|---|---|
| FT-IR | Detection of different bond and functional groups (aromatic and aliphatic compounds) | Both solids and solution sample can be analysed. Fast, easy, and sensitive. Overlapping spectra of NOM compounds makes interpretation difficult | (Lim *et al.*, 2018, Wan *et al.*, 2019, Nonfodji *et al.*, 2020, Okoro *et al.*, 2021) |

# 3 Overview of PCP types, biophysical properties, and mechanism of coagulation-flocculation

## 3.1 PCP types and their distribution

To date, several PCPs have been used for water clarification, some of which are cationic, anionic, polyanionic or non-ionic natured. These PCPs originate from more than 38 countries, including Asia, Africa, Europe and America and have different coagulating compounds which could be influenced by cultivation factors in location. This section provides an up-to-date summary of PCPs types and origin.

The Moringa genus comprises of 13 species (Verdcourt, 1985) out of which five (*Moringa oleifera* (MO), *Moringa stenopetala*, *Moringa peregrina*, *Moringa Drouhardii*, and *Moringa longitu*) have reportedly been used for CF water treatment due to their particle aggregation potentials (Megersa *et al.*, 2018). The *Moringa oleifera* (MO) belongs to the family *Moringaceae* and, although native to Africa and Asia, it is known for its drought tolerance, rapid growth rate and wide distribution. MO seed is the most researched PCP used in water treatment. MO seeds are globular, brown coloured, and may be winged or unwinged with oily cotyledons. Another promising PCP is the Hibiscus plant, a part of the *Malviceae* family that are well distributed worldwide, including Africa, Asia, the Middle East and the Southern part of America (Benchasri, 2012). *Malviceae* approximately have 88 genera and a specie distribution of 2300 (Burkill, 1997). One of the Hibiscus plant species, *Abelmoschus esculentus* also called *Hibiscus esculentus* (Okra), is widely distributed worldwide, especially the tropics.

374 It is known by its green colouration of the leaves and fruits, and its elongated seedpod which
375 encloses the seeds. The seeds and the mucilage are reported to have good NOM removal ability
376 (Jones and Bridgeman, 2016). *Hibiscus Cannabinus (*Kenaf), another Hibiscus family species,
377 is a herbaceous plant and is widely distributed in East, West and Central Africa (Jones and
378 Bridgeman, 2016). Kenaf has been recently used in water treatment research and, just like
379 Okra, has promising potential in water treatment (Okoro *et al.*, 2021). Russel (*Hibiscus*
380 *Sabdariffa*) is a plant native to India and tropical Africa (Dalziel and Elliot, 1973).

381 Several other plants have been used in water treatment: Nimali extract, an anionic,
382 polysaccharide and protein-containing plant from the *Loganiaceae* family common to Sri
383 Lanka and India, and reported to be the earliest plant used in water treatment over 4000 years
384 ago (Sen and Bulusu, 1962); Opuntia, a member of the *Cactaceae* family and well distributed
385 in Central America and most arid zones of the world (Diaz *et al.*, 1999, Zhang *et al.*, 2006)
386 with an approximate 1750 known species; thecommon bean (*Phaseolus vulgaris)* (Marobhe *et*
387 *al.*, 2007); *Luffa cylindrical* extract of the *Cucurbitaceae* family and common to Africa, Asia
388 and the United States of America (Anbukarasi and Kalaiselvam, 2015); Fava beans (*Vicia fava*
389 Linn L), a crop native to the Mediterranean and cultivated mainly in Europe and South America
390 (Mihailović *et al.*, 2010); Cocos nucifera fruit (coconut tree, a member of the palm tree family
391 (Arecaceae) and the only living species of the genus Cocos (Fatombi *et al.*, 2013); *Sterculia*
392 *scaphigerum* is native to South East Asia and has a coarse covering. *Cyamopsis tetragonoloba*
393 (Guar gum) (Priya *et al.*, 2017) and *Maerua subcordata*, are commonly used plants in Ethiopia
394 (Megersa *et al.*, 2018); *Margaritarea discoidea* is a tropical sub-Saharan Africa plant (Oladoja
395 *et al.*, 2017), with good coagulation potentials.

396 Most of these PCPs contain compounds like saponin, steroid ring, deoxy sugar, alkaloids,
397 tannins, phenolic and flavonoids, some of which possess coagulation and flocculation abilities.

The biophysical features of these PCPs, including their reported active coagulation components and CF mechanism, are further discussed in the sections below.

## 3.2 Biophysical properties of PCPs

### 3.2.1 Cationic PCPs

Cationic PCPs mostly comes from positively charged molecules such as the amine ($NH_4^+$) group attached to the molecule (see functional groups, Table 3). They neutralize the negatively charged electric double layer of the dispersed particles by Coulomb forces of attraction and saturation of differentially charged surfaces similar to the illustration by Okuda *et al.* (2001a) (Fig. 3). Some cationic PCPs have long polymeric chains which agglomerate particles by adsorbing onto the particle surface, then looping or tailing to another surface (Fig. 4).

The well-studied cationic PCP, -MO, contains cationic protein which has been designated as 2S albumin protein ($MO_{2x}$) (Ndabigengesere *et al.*, 1995, Miller *et al.*, 2008) and hemagglutinating protein (cMoL) (de Andrade Luz *et al.*, 2013). As shown in Table 3, MO has also been fractionated into Globulin, Albumin, Prolamin and Glutelin fractions, with Globulins and Albumins having the highest fractions of 53% and 44% respectively. MO has an isoelectric point (IEP) ranging from 10-11 (Ndabigengesere *et al.*, 1995, Ghebremichael *et al.*, 2005), and molecular weight (mW) between 6.5-66 kDa (Ghebremichael *et al.*, 2005, Baptista *et al.*, 2017). Although most studies have reported protein being the predominating coagulating agent (Okuda *et al.*, 2001a), several other organic compounds such as cationic peptides could be responsible for its coagulation performance. The mechanism of coagulation is well reported as a bridging and neutralisation mechanism (Table 3).

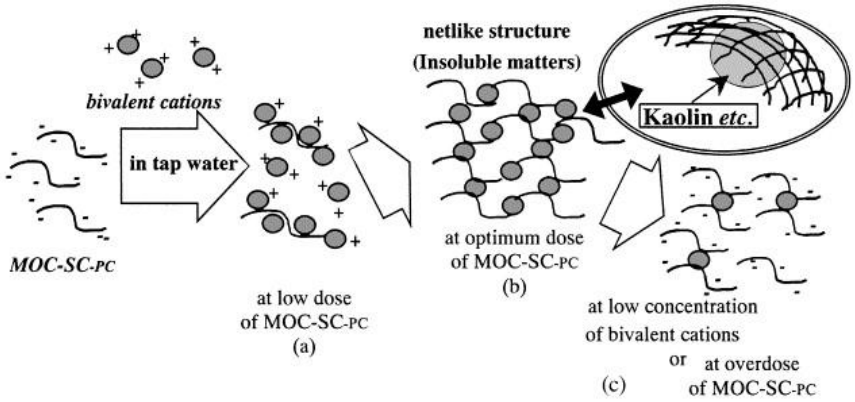



**Fig. 3 The proposed schematic representation of coagulation mechanism using purified salt extract (MOC-SC-pc) in kaolin suspension (Okuda *et al.*, 2001a)**

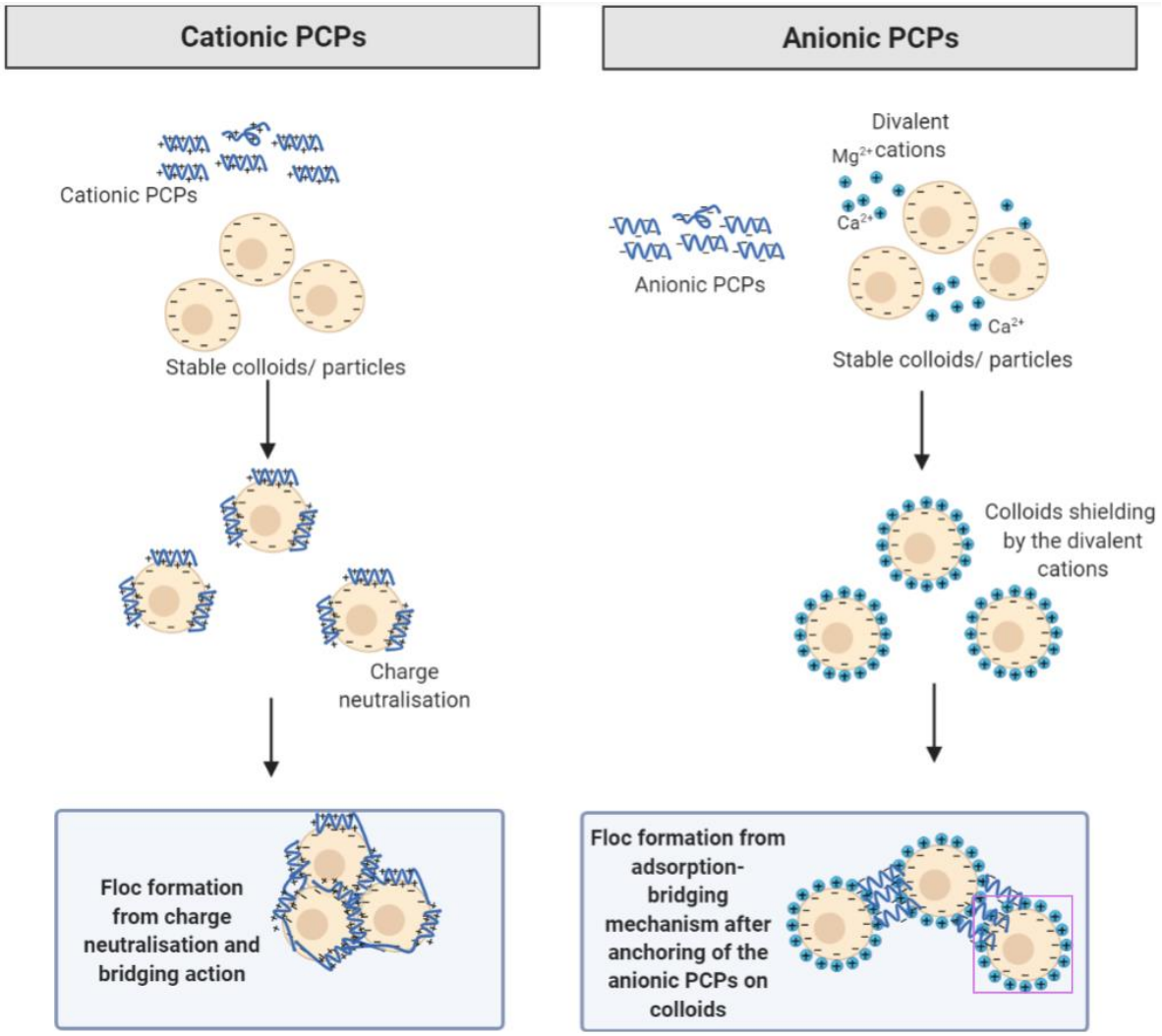



**Fig. 4 Mechanism of coagulation for cationic and anionic PCPs**

426 **Table 3 Selected biophysical properties of PCPs**

| Coagulant (PCP) Description | Coagulant type | Coagulant biophysical characteristics | Chemical compounds/ functional groups | Reference |
|---|---|---|---|---|
| *Abelmoschus esculentus* - Okra | Non-ionic | -Protein band between molecular weight (mW) 4-45kDa<br>-CF mechanism is Adsorption, bridging | -22% rhamnose, 25% galactose, 27% galacturonic acid and 11% amino acid -tryptophan-like, tyrosine-like, fulvic and humic acid-like peaks reported | (Mishra *et al.*, 2008, Jones and Bridgeman, 2016a) |
| *Brassica* specie (sp). seeds | Cationic | -mW of range 6.5-116 kDa; pI is 9.76; thermal stability at 95ºC<br>-CF mechanism is charge neutralisation, bridging | -Secondary protein with 19 amino acid residues including polar amino acids (8Gln and 1Thr) and positively charged amino acids (1 Arg and 1 His) | (Bodlund *et al.*, 2014) |
| *Ceratonia siliqua* (Carob gum) | Non-ionic | -mW ranging 5-8 kDa<br>-CF mechanism is adsorption-bridging | Mannose, galactose, and arabinose | (Kök, 2007, Haddarah *et al.*, 2014) |
| *Cocos nucifera* (Coconut) fruit | Cationic | -mW of 5.6 kDa, IEP of 7.5, CD of 1.09 meq/g<br>-CF mechanism is charge neutralisation, bridging | -Purified casein; primary, secondary and tertiary amide reactive groups. Active -OH and -NH sites reported | (Fatombi *et al.*, 2013) |
| Corn and Tapioca starch | Cationic | -Charge density (CD) 0.04 and 0.5 meq/g of polymer, respectively<br>-CF mechanism is charge neutralisation, bridging | C-amylose and amylopectin | (Cornwell and Brown, 2017) |
| *Cyamopsis tetragonoloba* (Guar gum) | Non-ionic | -mW range of 50-800 kDa<br>-CF mechanism is adsorption-bridging | Predominantly polysaccharide | (Priya *et al.*, 2017) |
| *Dolichos lablab* (Hyacinth bean) | Non-ionic | -Apparent masses ranging from 10 kDa to 67 kDa<br>-CF mechanism is adsorption-bridging | -Galactose and arabinose by ratio 2:1; glucose, xylose and mannose, lectin | (Mo *et al.*, 1999) |
| Fava beans (*Vicia faba* L.) | Poly-ionic | -Isoelectric point is at pH 4.04<br>-CF mechanism is charge neutralisation, bridging | -Protein (22.4-36.0%) and carbohydrate (57.8-61.0%) | (Mihailović *et al.*, 2010, Kukić *et al.*, 2015) |
| Hibiscus species including *Hibiscus rosa sinensis* leaf, *Hibiscus cannabinus* (kenaf) | Anionic | -mW between 7-75 kDa<br>-CF mechanism is adsorption-bridging | -Protein, hydroxyl, carboxyl and amines groups | (Mohamed *et al.*, 1995, Jones and Bridgeman, 2016b, Nidheesh *et al.*, 2017, Mariod *et al.*, 2017, Okoro *et al.*, 2021) |
| Industrial cationic starch, i.e., starch-graft-poly [2-methacryloyloxyethyl) trimethyl | Cationic | -Medium molecular weight and CD; Charge neutralisation, bridging<br>-CF mechanism is charge neutralisation, bridging | -Starch predominantly the hydroxyl group (O-H) | (Bolto and Gregory, 2007, Saleem and Bachmann, 2019) |

| | | | | |
|---|---|---|---|---|
| ammonium chloride (e.g., BORCET SZ 2000) | | | | |
| *Margaritarea discoidea* Fruit Seed Extract FSE | Non-ionic | -Polysaccharide differs in molecular weight, structure, and solubility<br>-CF mechanism is adsorption-bridging | -Galactomannans (galactose and monosaccharide mannose) and uronic acid (contains –CO and –COOH) | (Oladoja *et al.*, 2017) |
| *Moringa oleifera* (drumstick) seed | Cationic | -mW of extracted protein was 6.5kDa in reducing form while non reducing form was 13kDa; IEP between 10-11<br>-mW of isolated fractions were distributed between 0.9kDa-66kDa<br>-CF mechanism is charge attraction, charge neutralisation and bridging. | -2S albumin protein ($MO_{2x}$) and hemagglutinating protein (cMoL)<br>-Protein fractionated into Globulin, Albumin, Prolamin and Glutelin. Globulins (53% seed protein) and Albumins (44% of protein seed). | (Gassenschmidt *et al.*, 1995, Ndabigengesere *et al.*, 1995, de Andrade Luz *et al.*, 2013, Baptista *et al.*, 2017) |
| Nirmali seeds *Strychnos potatorum* | Non-ionic | -Isolated protein had mW of 12kDa<br>-CF mechanism is adsorption-bridging | -Protein (about 89µg/mL) and polysaccharide (14.3% of seed); galactan and galactomannan | (Vijayaraghavan *et al.*, 2011, Arunkumar *et al.*, 2018) |
| *Opuntia focus-indica* (nopal) | Anionic/ Non-ionic | -IEP ~2; pKa 9.0 ± 0.6<br>-CF mechanism is adsorption-bridging mechanism | -Highly branched carbohydrate polymer including arabinose, galactose, rhamnose, galacturonic acid, and xylose | (Matsuhiro *et al.*, 2006, Miller *et al.*, 2008, Bouaouine *et al.*, 2018) |
| *Phaseolus vulgaris* (common bean) | Anionic | -mW ranging 26-50kDa<br>-CF mechanism is adsorption, bridging | >50% of globulin protein | (Morales-de León *et al.*, 2007, Antov *et al.*, 2010) |
| *Scaphium scaphigerum* (Malva nut) seed gums | Non-ionic | -mW of $6.65x10^6$ Da; intrinsic viscosity (dl/g) of 10.0, and polydispersity index of 1.1<br>-CF mechanism is adsorption-bridging | -62.0% total carbohydrates plus 8.3% proteins; 31.9% arabinose, 29.5% rhamnose, 29.2% galactose, 6.4% uronic acid; glucose, xylose and mannose | (Somboonpanyakul *et al.*, 2006) |
| *Tamarindus indica* ssed gum (Tamarind) | Non-ionic | -mW ranging 700-880 kDa<br>-CF mechanism is adsorption-bridging | -Neutral sugars - glucose, xylose and galactose | (Kaur *et al.*, 2012) |
| *Trigonella foenum-graecum* (Fenugreek gum) | Non-ionic | -mW of 32.3 kDa<br>-CF mechanism is charge neutralisation, inter-particle bridging | -Protein, ketone, carbonyl, carboxyl, aliphatic xylan, polysaccharides and coumarin | (Jiang *et al.*, 2007, ELsayed *et al.*, 2020, Kim *et al.*, 2020) |
| *Vigna unguiculata* (cowpea) | Cationic | -mW of approximately 6 kDa; main mW reported between 23-52 kDa; isoelectric precipitation pH 4.5<br>-CF mechanism is charge neutralisation, bridging | -Soluble protein | (Rangel *et al.*, 2003, Marobhe *et al.*, 2007) |

427

Another cationic PCP is *Cocos nucifera* (Coconut) fruit, a drupe that contains casein, a protein-based compound. The purified casein has a mW of 5.6 kDa, IEP of 7.5, charge density of 1.09 meq/g (Table 3). It contains the reactive amide groups, hydroxy group (alcohols and carboxylic acids) and the amino group, as shown in Fig. 5 (Fatombi *et al.*, 2013). Coconut fruit is reported cationic due to the presence of a dominant amide group. Other cationic PCPs include *Vigna unguiculate* seed proteins having mW of approximately 6 kDa (Marobhe *et al.*, 2007), *Brassica* specie (sp). Seeds, with a mW of isolated cationic protein (amino acids) in the range 6.5-116 kDa (Bodlund *et al.*, 2014), *Parkinsonia aculeate* (Marobhe *et al.*, 2007), *Plantago ovata* (also called psyllium Indian) (Ramavandi, 2014) and some starches (Choy *et al.*, 2016, Abdo *et al.*, 2020) are also cationic.

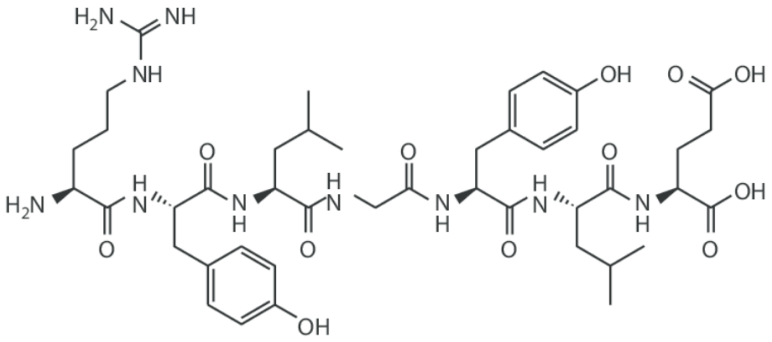
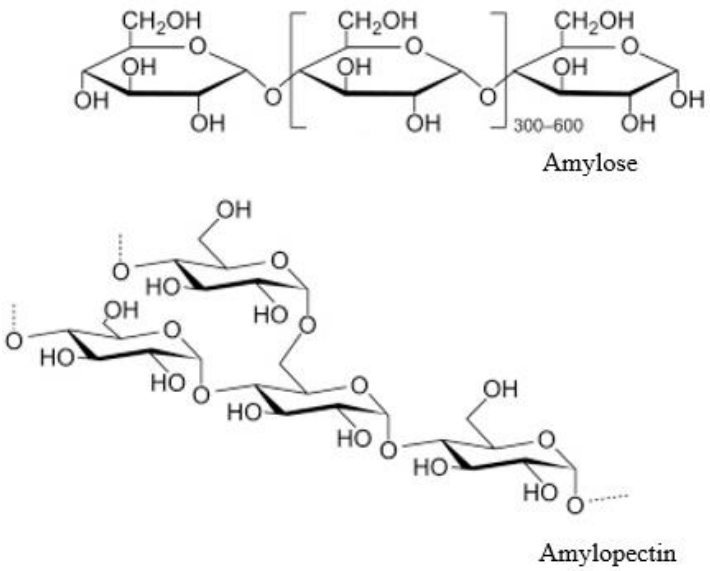


**Fig. 5 Molecular structure of casein ((Reddy *et al.*, 2016))**

The starch present in seeds, tubers, fruits, stems and leaves of most cereal grains, cassava, potato, banana, sago and maize plants, consists of two polysaccharides: c-amylose and amylopectin (Cornwell and Brown, 2017, Saritha *et al.*, 2017). Amylopectin has a high mW > $10^8$ g/mol, making a suitable flocculant aid (Cornwell and Brown, 2017). Starches (Fig. 6) generally have poor NOM removal performance due to their low cationic charge density (corn and tapioca starch are 0.04 and 0.5 meq/g of polymer (Cornwell and Brown, 2017). Modifying these starches overcomes this limitation and makes them suitable for the intended use. Cationic starch can be modified through a gelatinization process in the presence of a base. Structural

448 modification of starch can also be through graft polymerisation (Liu *et al.*, 2017, Chua *et al.*,
449 2020). Polyacrylamide (PAM) (Ma *et al.*, 2017) has also been used for grafting. Examples of
450 structurally modified starch include BORCET SZ 2000 (derived from potato starch and has
451 about 20% solubility in aqueous medium) (Ziółkowska and Shyichuk, 2011).




452

453 **Fig. 6 Molecular structure of water-soluble polysaccharides (a) amylose, (b) amylopectin, found**
454 **in PCPs containing starch. Adapted from (Saritha *et al.*, 2017)**

455 **3.2.2 Anionic PCPs**

456 Removal of NOM by anionic PCPs is generally through the bridging process facilitated by
457 particle adsorption (Table 3, Fig. 4). During this process, compounds present in the anionic
458 PCP forms complexes with the NOM and some bivalent cations present in the solution,
459 resulting in floc formation. Anionic PCPs bind onto NOM by covalent bond formation
460 (Oladoja, 2015, Okoro *et al.*, 2021). An anionic PCP, *Phaseolus vulgaris* (also called common
461 bean seed) predominantly contains >50% of globulin protein and has mW ranging 26-50kDa
462 (Morales-de León *et al.*, 2007, Antov *et al.*, 2010).

463 Tannins are polyphenols and generally an anionic polymer present in almost every PCP, with
464 some having substantial quantities (Özacar and Şengil, 2003). They are chemically complex

and consists of two main groups, namely the condensed tannins (derivatives of flavanols) (Fig. 7a), and the hydrolysable tannins (esters of a sugar, usually glucose, Fig. 7b) (Özacar and Şengil, 2003). The effectiveness of tannins as coagulants mainly depends on their chemical structure and degree of modification (Hemingway and Karchesy, 2012). Some tannins, such as tannic acid, resorcinol, pyrogallol, catechin, and several hydroxyphenyl groups, have high molecular mass, giving them an ampholytic property necessary for binding both anionic and cationic molecules. Some tannins such as common oak (*Quercus robur*) are anionic at higher pH (Oladoja, 2015), resulting in their bivalent cationic attraction, which then causes particle enmeshment over time and thus removal. Other anionic tannins reportedly used in water treatment are Quercus *macrolepis* (acorns-Valonia) (Özacar and Şengil, 2002), and crude forms of horse chestnut (*Aesculus hippocastanum*) (Šćiban *et al.*, 2009), Acacia, *Castanea sativa*, Schinopsis, corn cup of *Quercus ithaburensis macrolepis* (Valonia oak) (Saleem and Bachmann, 2019). Tannins can be modified to improve NOM removal through the jellification, cationization and etherification process. The jellification process involves tuning of the tannin structure to remove cationic NOM optimally. Another modification process is cationization which improves binding to negatively charged NOM. More information on these processes is available in the literature (Quamme and Kemp, 1985, Reed and Finck, 1997, Mitchell *et al.*, 1998).

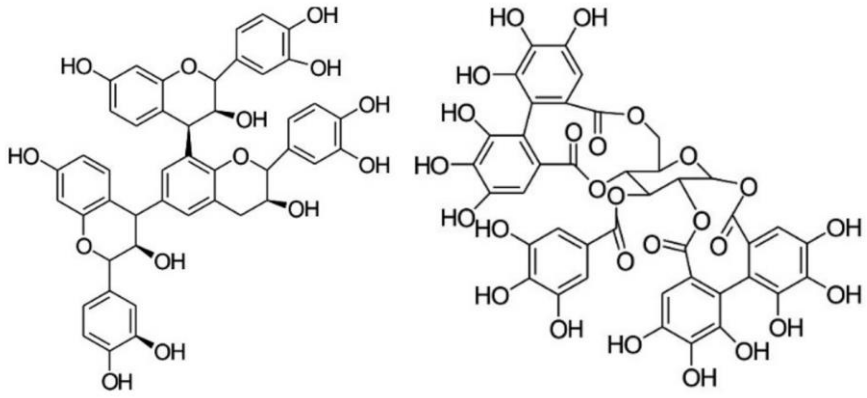


**Fig. 7 Molecular structures of (a) condensed tannin (b) hydrolysable tannin (adapted from (Raja *et al.*, 2014))**

Mucilage present in *Opuntia focus-indica* (nopal) is an anionic, highly branched carbohydrate polymer. It contains arabinose, galactose, rhamnose, galacturonic acid, and xylose (Fig. 8) (Matsuhiro *et al.*, 2006). It has IEP of ~2, which indicates that predominant surface charges are negative. Coagulation behaviour is by the adsorption-bridging mechanism resulting from dipole interaction of mucilage with divalent cations present in the Opuntia spp. (Matsuhiro *et al.*, 2006).

Hibiscus plants such as *Hibiscus cannabinus* (kenaf), *Hibiscus sabdariffa* (roselle) and *Hibiscus rosa-sinensis*, are anionic polymers (Awang and Aziz, 2012, Okoro *et al.*, 2021) containing hydroxyl, carboxyl and amines functional groups (Nidheesh *et al.*, 2017). They have a molecular weight ranging between 7-75 kDa (Mariod *et al.*, 2017) with similar anionic coagulation mechanism as the Opuntia spp.

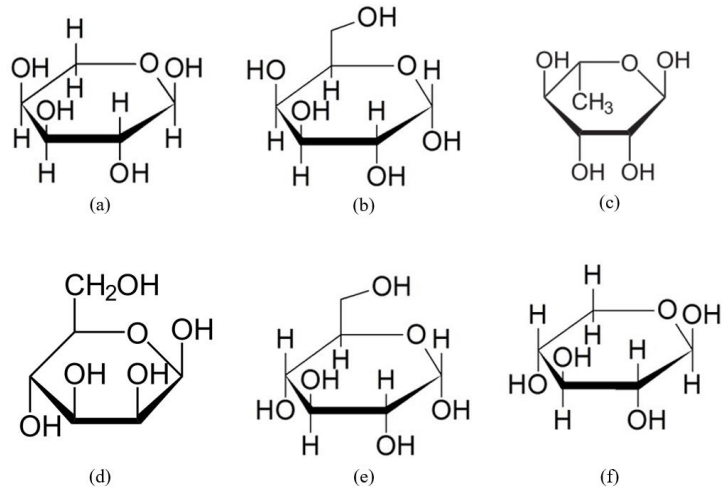



**Fig. 8 Molecular structures of (a) arabinose, (b) galactose, (c) rhamnose (d) mannose, (e) glucose, (f) xylose (adapted from (Wiercigroch *et al.*, 2017))**

### 3.2.3 Non-ionic and poly-ionic PCPs

Polymeric molecules with net-zero charges are non-ionic. These PCPs contain polysaccharide compounds (mostly found in gums) such as amylopectin (Fig. 6, Table 3) (Adinolfi *et al.*, 1994, Choy *et al.*, 2015). They have adhesive properties that aid their binding to surfaces of NOM. Plant gums have broadly two categories, i.e., the galactomannans and non-galactomannans. Galactomannans are polysaccharides with a mannose backbone and galactose side groups (Adinolfi *et al.*, 1994). Examples of plants belonging to this group are: *Cyamopsis tetragonoloba* (Guar gum) having mW range of 50-800 kDa (Priya *et al.*, 2017) and mostly polysaccharide; *Ceratonia siliqua* (Carob gum) with mW ranging 5-8 kDa (Haddarah *et al.*, 2014); Prosopis species such as *Prosopis juliflora* and *Prosopis larvigata* with mW of about 62 kDa (Saleem and Bachmann, 2019); *Trigonella foenum-graecum* (Fenugreek gum) with mW of 32.3 kDa (Jiang *et al.*, 2007); *Strychnos potatorum* (clearing-nut gum) (Adinolfi *et al.*, 1994). These PCPs differ in their mannose to galactose ratio and their monosaccharides. The coagulation compounds present in *Margaritarea discoidea* are predominantly galactomannans (polysaccharides, containing galactose and monosaccharide mannose (**Fig.** 8), that is non-ionic and water-soluble) and uronic acid (contains –CO and –COOH) (Oladoja *et al.*, 2017). The non-ionic nature of the PCP prevents ion pairing to the solution particles, suggesting that the CF mechanism is by the adsorption-bridging process. *Sterculia scaphigerum* (Malva nut gum, a type of seed gum) contains carbonyl and carboxylic acid functional groups present in uronic acid (Somboonpanyakul *et al.*, 2006). The CF mechanism of *Sterculia scaphigerum* is by particle bridging facilitated by adsorption.

Non-galactomannans lack the mannose monosaccharides in their backbone. Examples of non-galactomannans are *Abelmoschus esculentus* (Okra), containing 22% rhamnose, 25% galactose, 27% galacturonic acid and 11% amino acid (Mishra *et al.*, 2008), *Dolichos lablab* (Hyacinth bean), containing galactose and arabinose by ratio 2:1 and with 6% of its components

found in uronic acid (glucose, xylose and mannose) (Mo *et al.*, 1999), *Opuntia ficus-indica* (also called Barbary fig gum) (Miller *et al.*, 2008), containing galactose, arabinose, rhamnose, uronic acid, and *Tamarindus indica* (Tamarind) containing neutral sugars with mW ranging 700-880 kDa (Kaur *et al.*, 2012).

Fava (or broad) bean PCP (*Vicia fava* Linn L), a poly-ionic PCP, has high protein (22.4-36%) and carbohydrate (57.8-61%) content (Mihailović *et al.*, 2010, Kukić *et al.*, 2015). Its isoelectric point is at pH 4.04, and its surface is predominantly poly-ionic (different electrical charge). Great progress has been made in the identification of PCPs's biophysical properties. However, the biophysical properties of some PCPs which defines their coagulation compounds and their properties, are yet to be analysed. So, future studies should focus on characterising and identifying these polymeric coagulating compounds using appropriate techniques.

Table 4 Overview of advantages and shortcomings of PCPs

| Item | Effectiveness | Drawback |
|---|---|---|
| Reliability | -Most PCPs do not modify the water pH, so CF treatment do not require alkalizing products such as sodium or calcium hydroxide. | -Shorter shelf life due to presence of degradable biological substances (Ghebremichael *et al.*, 2006, Choy *et al.*, 2015, Baptista *et al.*, 2015, Camacho *et al.*, 2017)<br>-Some PCPs such as MO may be less effective in more acidic or basic water (Ferrari *et al.*, 2016, Gautam and Saini, 2020); reported change in water pH after treatment by *Opuntia ficus indica* |
| Cost and management | -Some are potentially inexpensive for small-medium scale application: they use little or no technology and may not require the use of flocculants. | -Some PCPs are moderately effective and can only be used as CF aids. However, this problem is currently being addressed through the graft polymerisation process. (Pal *et al.*, 2012, Sun *et al.*, 2016, Chua *et al.*, 2020).<br>-May not be cost effective and sustainable for large-scale treatment because of dosage volume needed for treatment which |

| | | |
|---|---|---|
| | | is higher than conventional coagulants. In addition to their high dosage volume, they require bigger climate-controlled storages. |
| Environmental Health | -Consists of natural and sustainable compounds making them environmentally friendly and possess low carbon footprint. PCPs are not corrosive or dangerous to health (Bolto and Gregory, 2007) | -PCPs adds nutrients to treated water potentially posing a risk of by-products formation harmful to human health. |
| Post treatment application and by-products use | -Generate low sludge volume and the produced sludge are biodegradable and valuable for instance, in agriculture (Renault *et al.*, 2009) | -NA- |
| Accessibility | -Easily available to all including remote locations where conventional coagulants would otherwise have been difficult to acquire. | -PCPs have diverse applications implying that their increased demand by water industry would influence their accessibility for other purposes, which may affect their costs. For instance, *M. oleifera* is a major food crop in Africa and Asia, and is used for drug manufacturing; Okra plant is used as a vegetable in Western Africa and Southeast Asia, fuel, paper manufacturing in Malaysia (Posmontier, 2011, Roy *et al.*, 2014, Singh *et al.*, 2014, Terkula *et al.*, 2021). So, to guarantee wide application, and sustainable use.<br><br>-Seasonal variation: PCPs do not grow all-year round raising concern about their availability. Sourcing a non-competitive PCPs that is local to an environment may solve potential accessibility problems. |

Selecting a suitable PCP begins with understanding the factors influencing the CF process previously illustrated in Fig. 2, and the PCP's biophysical properties (see Section 3.2, Table 3). The first step taken during any water treatment process is to examine the water characteristics such as the pH, alkalinity, temperature, presence of co-existing ions and solids content, and then choose a suitable coagulant to fulfil a treatment objective. The water

542 characteristics, PCPs biophysical properties and the treatment system conditions such as
543 mixing speed, are interrelated (Bolto and Gregory, 2007), and should be well understood.
544 Biophysical properties such as the CD helps to determine the optimum dosage when a charge
545 neutralisation mechanism prevails, while a high mW is essential for a bridging mechanism
546 (Gregory, 1998, Gregory, 2013). Further details on NOM removal performance of some PCPs
547 are discussed in Section 5.

548 Confirming the PCP type and optimum dosage is usually determined by jar tests (Hudson and
549 Wagner, 1981), and other techniques such as SUVA and fluorescence spectroscopy, which can
550 provide specific information such as organic matter content, vital for meeting certain treatment
551 objectives (tools are further discussed in Section 4). To ensure sustainable use, it is important
552 that the selected PCP is easily accessible, stable during storage duration, and easily processed
553 (Table 4). For drinking water use, the selected PCPs must satisfy the health and quality
554 guideline, otherwise it may be suitable for use as a coagulant aid or for wastewater treatment.

555 # 4 Methods used for estimating and characterising OM, floc formation and behaviour
556 in PCPs coagulated water

557 ## 4.1 Characterisation of PCPs structural properties and functional groups

558 NOM and the organic molecules present in PCPs share many similar properties, although they
559 are different. Several physical and chemical NOM fractionation techniques can produce pure
560 components needed for these characterisation procedures. The physical fractionation methods
561 are electrophoresis, size exclusion chromatography (SEC), ultracentrifugation and
562 ultrafiltration (Chow *et al.*, 2005a, Baptista *et al.*, 2015). The chemical methods include
563 adsorption chromatography, precipitation, and solvent extraction (Arunkumar *et al.*, 2018,
564 Okoro *et al.*, 2021). These characterisation techniques have barely been used in PCP studies,
565 probably due to a lack of access, resulting in limited information which could further improve

566 understanding of the PCPs performance during the CF water treatment process. One of the
567 simplest procedures is the Elemental Analyser which estimates the concentration of elements
568 such as carbon, nitrogen and oxygen in the samples (Priya *et al.*, 2017). Information from
569 elemental analysis can be used to determine the structure of an unknown compound in the
570 PCPs. Similarly, the Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP AES)
571 can also determine the elemental composition, although it has not been used in PCPs study
572 (Matilainen *et al.*, 2011).

573 Characterization of NOM using the ultrafiltration (UF) membrane technologies involves
574 physically segregating the particles based on their molecular weight; this process is pressure-
575 driven (Baptista *et al.*, 2015) and can handle a large quantity of MO samples (Baptista *et al.*,
576 2015). However, it is known to produce inconsistent fractions of NOM due to the variation in
577 membrane sizes, mostly from particles. The XAD resins characterize NOM based on their
578 hydrophobic acid (HPO) and hydrophilic acid (HPI) fraction. The Amberlite XAD resin
579 differentiates fractions into bulk hydrophobic acid (HPO), hydrophilic acid (HPI) and
580 hydrophilic neutral fractions (HPI- neutral) using the XAD-8 absorbable or the XAD-4
581 absorbable resin accordingly (Malcolm and MacCarthy, 1992). However, this technique has
582 been reported to poorly recover HPI NOM fraction in water (Tian *et al.*, 2018).
583
584 Another tool used in characterizing NOM is the high-performance size exclusion
585 chromatography (HPSEC), a high-pressure operating system that requires a small volume of
586 sample for operation. Differentiation of molecular particle size is by a controlled pore size
587 porous gel (Pelekani and Snoeyink, 1999). When molecules meet the gel pore, they get rejected
588 due to their inability to go through pores (Chow *et al.*, 2005b). Although this technique
589 experiences the same drawback for the UF membrane method, i.e. performance mostly

restricted to smaller particle sizes and molecular insolubility, it has been reported to be a proven tool for studying NOM during water treatment *Opuntia ficus indica* (Bouaouine *et al.*, 2019).

Fourier-transform infrared spectroscopy (FT-IR) operates using absorbed energy from the transmitted infrared light corresponding to the atomic bond's vibrational energy (Matilainen *et al.*, 2011). The FT-IR absorption spectrum produced is a unique fingerprint of compounds present in the sample (Table 5), which shows the organic and inorganic functional groups (Bouaouine *et al.*, 2018, Okoro *et al.*, 2021). Another characterisation method is the Circular dichroism (CD), an absorption spectroscopy, which uses polarised light (circular) to classify compounds and estimate their α-helix content (Kwaambwa and Maikokera, 2008). CD spectra signatures provide information on the secondary structure of the coagulating protein in the form of α-helices. Fewer α-helix content is attributed to weak cationic coagulant whereas, higher α-helix content indicates a stronger cationic coagulant (Kelly *et al.*, 2005). CD technique has successfully been used to characterise MO PCPs (Suarez *et al.*, 2005, Kwaambwa and Maikokera, 2007, Kwaambwa and Maikokera, 2008, Nordmark *et al.*, 2016), and it can be noted that CD use can clearly illustrates structure of PCPs, and potentially their interactions with metals and other particles in suspension. Data on CD analysis of other PCPS is scarce, and thus encouraged to provide information on their structure and understand their CF mechanism. Both the FT-IR and CD spectroscopy are a complementary technique for investigating the structure of the PCPs. The ionic strength of a solution, including pH and surfactants concentration, can affect the structure and conformation of coagulating compound.

**Table 5 Selected FT-IR spectra obtained from PCPs coagulated water samples vis-à-vis *Opuntia ficus-indica*. Adapted from (Nharingo and Moyo, 2016, Bouaouine *et al.*, 2018, Gandiwa *et al.*, 2020)**

| Band ($cm^{-1}$) | Assignment | Functional groups/ chemical compounds |
|---|---|---|
| >3100 | O-H stretch | Alcohols, carboxylic and phenols groups |

| | | |
|---|---|---|
| 3000 | -OH, -NH | Carboxylic acid, amino acid, alcohol |
| 2850-2960 | C-H stretch ($CH_3$ and $CH_2$) | Alkanes, carboxylic acid |
| 2850 | -$CH_2$ stretching | Carbonyl group |
| 2620 | O-H stretch | Hydrogen-bonded and carboxylic groups |
| 1713-1720 | C=O stretch | Carboxylic groups |
| 1658 | -$CONH_2$ | Proteins |
| 1618, 1630 | C=C stretch | Alkenes and aromatic rings |
| 1540, 1574 | N-H bend and deformation | N-H structures, amines |
| 1455 | C-H bend ($CH_3$ and $CH_2$) | Alkanes, carboxylic acid |
| 1430 | O-H stretching | Phenols |
| | C=O stretching | Carbohydrates |
| 1410 | O-H bend | Carboxylic groups |
| 1375 | C-H bend ($CH_3$) | Alkanes |
| 1321 | $ArNH_2$ and CN stretch | Primary aromatic amines |
| 1260, 1250 and 1220 | C-O stretch | Aromatic, carboxylic and phenol groups |
| 1231 | P=O | Phosphates |
| 1072 | C-O-C and OH | Polysaccharides |
| 1095 and 1030 | C-O stretch | Alcohols and aliphatic ethers |
| 1041 | HC-O-H | Cyclic alcohols |
| 1027 | R-$CH_2$-OH | Glucose units on PCPs |
| <1000 | C-H bend and aromatics | Tri- and tetra substitutes aromatic rings; aromatic groups |

Large complex molecules present in the PCP coagulated water can be pyrolysed, and their functional groups detected using gas chromatography-mass spectrometry (GC–MS). It is a useful technique that can produce comprehensive structural information on the molecular building block of a PCPs (Matilainen *et al.*, 2011). Solid-state 13C NMR provides important information on carbon structures present in the coagulated water sample (Adinolfi *et al.*, 1994, Jiang *et al.*, 2007). This technique is advantageous when combined with elemental composition data, FT-IR or apparent molecular weight data. Other techniques scarcely reported include the Liquid chromatography-mass spectrometry (LC-MS), which combines the physical separation (HPLC) and mass analysis (MS) (Adinolfi *et al.*, 1994).

## 4.2 UV/Vis spectroscopy

The UV/Vis spectroscopy technique has routinely been used in PCP water treatment studies to quantify light-absorbing molecules present in the water and specific electromagnetic spectrum regions. The absorption spectrum indicates NOM concentration in the water and is influenced by the solution pH, turbidity, solvent used for PCP extraction, temperature, and any interfering chemical compounds or impurities present (Beaven and Holiday, 1952). NOM measurement is based on Beer's law. It is done using a UV/Vis spectrophotometer operating on a single wavelength or over a spectrum range. The range 220-280nm has mostly been used to characterise both NOM and PCPs chromophores (Beaven and Holiday, 1952). The absorbance value obtained from the instrument is based on the transmittance ($I/I_0$), which is defined as the ratio of light intensity after (I) and before ($I_0$) passing through the PCP coagulated water in the cuvette, according to Eqn. (1).

$$\boldsymbol{A = -log\left(\frac{\%T}{100}\right)} \qquad \textbf{Eqn. (1)}$$

Table 6, indicates the different spectroscopic indices (wavelength) used in characterising NOM. They provide an approximate estimate of various aromatic compounds found in water and PCPs (Baptista *et al.*, 2015, Okoro *et al.*, 2021)}. Some of the aromatic compounds include phenolic, carboxylic chromophores and polyhydroxy aromatic (PHA) moiety.

**Table 6 Spectroscopic indices for NOM classification (adapted from (Priya *et al.*, 2017))**

| UV spectrophotometric indices | Importance | References |
|---|---|---|
| 254 | Indicates the presence of aromatic groups and correlated with NOM reactivity | (Korshin *et al.*, 2009) |

| | | |
|---|---|---|
| 220 | Shows that carboxylic and aromatic chromophores exist | (Korshin *et al.*, 2009) |
| 272 | Shows reactivity of aromatic groups and could indicate the presence of chloroform | (Korshin *et al.*, 2009) |
| 253/203 | Indicates disinfection by-product formation potential (DBPFP) | (Kim and Yu, 2005) |
| 254/202 | It reflects the degree of activation of polyhydroxy aromatic (PHA) moiety in the coagulated water. Its coagulability shows DBPFP | (Korshin *et al.*, 2009) |
| Absorbance slope index (ASI), see Eqn. (2) | Indicates NOM reactivity and THMFP | (Korshin *et al.*, 2009) |
| 260 | Shows relative abundance of aromatic (C=C) fraction of NOM | (Chen *et al.*, 2002) |
| 465/665 | Shows relative abundance of aromatic (C=C) and ketonic (C=O) fraction of NOM | (Chen *et al.*, 2002) |
| 254/203 | Indicates reactivity of aromatic rings with the hydroxyl, carboxyl, and ester groups | (Ng *et al.*, 2013) |
| 280 | Indicates the presence of phenolic groups | (Chin *et al.*, 1994) |

Another useful NOM spectroscopy measurement technique is the absorbance slope index (ASI) and differential spectroscopy (ΔUV/Vis). ASI indicates the reactivity of aromatic groups present in the coagulated water. It also explains the apparent molecular weight fraction of organics present (Korshin *et al.*, 2009). Higher ASI values show poor removal of ASI from water enriched with an aromatic fraction of NOM. ASI value can be obtained from Eqn. (2)

$$\boldsymbol{ASI = 0.56\left(\frac{A_{254}-A_{272}}{A_{220}-A_{230}}\right)} \qquad \textbf{Eqn. (2)}$$

where UV absorbance at wavelength 254 nm, 272 nm, 220 nm and 230 nm are represented by $A_{254}$ nm, $A_{272}$ nm, $A_{220}$ nm and $A_{230}$ nm respectively.

Differentially absorbance ($\Delta A_{272}$ nm), which defines the absorbance change under certain conditions such as a change in coagulant and halogen dose (Roccaro and Vagliasindi, 2009), can illustrate the chlorine consumption behaviour of PCPs. Some of the PCP studies have reported using these spectroscopic techniques for estimating NOM concentration (Moreti *et al.*, 2016, Priya *et al.*, 2017). The removal of these absorbance indices illustrates the ability of PCPs to reduce DBPs precursors which can be an effective way of controlling trihalomethanes in water.

### 4.3 NOM bulk parameters: total organic carbon (TOC)/ dissolved organic carbon (DOC) and SUVA

Untreated water consists of organic and inorganic particles formed from natural or synthetic processes (Ezeabasili *et al.*, 2015, Santschi *et al.*, 2017). The NOM present in the untreated water have different water affinity. The hydrophilic (HPI) NOM fraction such as carbohydrate, proteins, carboxylic acids etc are more difficult to remove during treatment processes than their hydrophobic fraction (HPO); HPI fraction mostly comprises of fulvic (FA) and humic acid (HA) due to their light weight molecular mass and high biodegradability (Nkambule *et al.*, 2012).

Total organic carbon (TOC, mg/L) measures the combined organic contamination in a sample. The dissolved organic carbon (DOC, mg/L) is a measure of the dissolved organic carbon content and excludes suspended particles. Unlike the TOC, DOC measurement occurs after filtering samples through a 0.45 µm filter. Both parameters can characterise NOM during CF treatment using PCPs (Moreti *et al.*, 2016). The specific ultraviolet absorbance (SUVA, L/mg-m) is obtained by normalising the UV value to the sample's DOC concentration (mg/L). The

SUVA value indicates the aromaticity of the NOM content of water (Matilainen *et al.*, 2011, Okoro *et al.*, 2021). A sample with a high DOC value can raise UV absorbance, which would result in higher SUVA value. By normalising with the DOC value, the SUVA measurement reduces the aromatic biasness from the UV measurement values and presents a more realistic NOM characteristic of the water (Matilainen *et al.*, 2011).

$$SUVA\ \left(\frac{L}{mg.m}\right) = \left(\frac{UV_{254}\ (cm^{-1})}{DOC\ \frac{mg}{L}}\right)\ x\ 100\ \left(\frac{cm}{m}\right) \qquad \textbf{Eqn. (3)}$$

**Table 7 Typical SUVA values found in PCP coagulated water (Edzwald and Tobiason, 1999)**

| SUVA ($Lmg^{-1}m^{-1}$) | Composition |
|---|---|
| > 4 | Mostly aquatic humics, high hydrophobicity, high molecular weight |
| 2 – 4 | A mixture of aquatic humics and other NOM, a mixture of hydrophobic and hydrophilic NOM, a mixture of molecular weights |
| < 2 | Mostly non – humics, low hydrophobicity, low molecular weight |

High SUVA of coagulated water indicates a high concentration of humic substances in water and may also imply the coagulant's high nutrient content. Table 4 shows that SUVA value > 4 indicates aromatic, hydrophobic, and high chlorine demand compounds, while SUVA value < 3 shows that the compounds are mainly hydrophilic with low chlorine consumption. Most of the evidence to date has found a significance association between NOM removal during CF treatment and a high $SUVA_{254}$-value (Archer and Singer, 2006, Baptista *et al.*, 2015, Priya *et al.*, 2017).

In addition, correlation between DBP formation potential and $SUVA_{254}$ have been shown to strongly depend on the $SUVA_{254}$ status of the water (Hua *et al.*, 2020). It is without doubt that the PCPs coagulated water have a diverse mix of compounds which are both aromatic and non-

aromatic (Okuda *et al.*, 2001b, Okuda *et al.*, 2001a, Choy *et al.*, 2015, Saleem and Bachmann, 2019, Okoro *et al.*, 2021). Non -aromatic compounds such as some proteins and ketones, may poorly correlate with DBP formation because of their weak UV-absorption ability (Ates *et al.*, 2007, Hua *et al.*, 2018), whereas a good linear correlation of $SUVA_{254}$ and DBP formation potential is likely to exist for aromatic fraction of the PCPs (Jung and Son, 2008). Currently though, it is not clear whether this relationship applies to all PCPs due to limited information. In light of the contribution of the PCPs to the organic matter content of the treated water, a better understanding of the SUVA relationship with DBP formation in PCPs coagulated water can benefit the monitoring of DBPs.

### 4.4 Fluorescence spectroscopy

This analytical method analyses fluorescence from a sample using a light beam such as UV light, causing molecular excitement in certain compounds, resulting in their emitting light. Compared to UV/Vis, the fluorescence method has the advantage of rapid molecular prediction speed, better sensitivity and online molecular prediction capability (Bieroza, 2010) reported to be between 100-1000 times higher than the UV/Vis techniques (Guilbault, 2020). It is a well-established and reported NOM characterisation technique and has been used to identify the different fluorophores found in PCPs studies. Fluorophores represent the several aromatic groups that re-emit light upon excitation and are reported as excitation and emission wavelengths (Coble, 1996). The excitation-emission matrix (EEM) illustrates the structural components of the NOM.

**Table 8 Fluorescence EEMs peaks found in natural waters (Coble, 1996)**

| Peaks description | | Excitation wavelength (nm) | Emission wavelength (nm) |
|---|---|---|---|
| Humic substances | A | 237 – 260 | 400 – 500 |

| | | | |
|---|---|---|---|
| Humic substances | C | 300 – 370 | 400 – 500 |
| (highly coloured) | $C_1$ | 320 – 340 | 410 – 430 |
| | $C_2$ | 370 – 390 | 460 – 480 |
| Tyrosine – like protein | $B_1$ | 225 – 237 | 309 – 321 |
| | $B_2$ | 275 | 310 |
| Tryptophan – like protein | $T_1$ | 275 | 340 |
| | $T_2$ | 225 – 237 | 340 – 381 |
| Humic substances from marine | M | 290 – 310 | 370 – 410 |

Different EEM ranges (~200 to ~500 nm) have been used in visualising the different fluorophores present in water. Molecules present in the coagulated water are usually described by peaks such as humic-like, fulvic-like, protein-like. These peaks can be analysed by peak picking technique (retrieving independent peak position) or regional fluorescence integration (FRI) (peaks grouped into regions I-V) (Chen *et al.*, 2003). Other methods include advanced statistical and modelling tools such as multivariate analytical techniques (for example, principal component analysis - PCA, parallel factor analysis - PARAFAC, multiple linear regression methods) and modelling tools such as artificial neural networks (Bieroza, 2010). Very few studies have used the fluorescence EEM technique to characterise NOM in PCP coagulated water sample. Researchers have reported the presence of prominent fluorescent peaks (Table 8), which are associated with fluorophores such as tryptophan-like, tyrosine-like substances in MO (Kwaambwa and Maikokera, 2007), Hibiscus plants products (Jones and Bridgeman, 2016a), and a cationized starch (Liu *et al.*, 2017). This technique can provide information on the relative concentration of fluorescent organic matter fractions in such ranges, as shown in Table 8. The fluorescence technique shows the presence of NOM in the source

water and signifies when coagulating compounds in PCPs leach into the coagulated water (Fig. 9). Thus, it can be a handy tool for monitoring compounds' contribution to DOC of water.

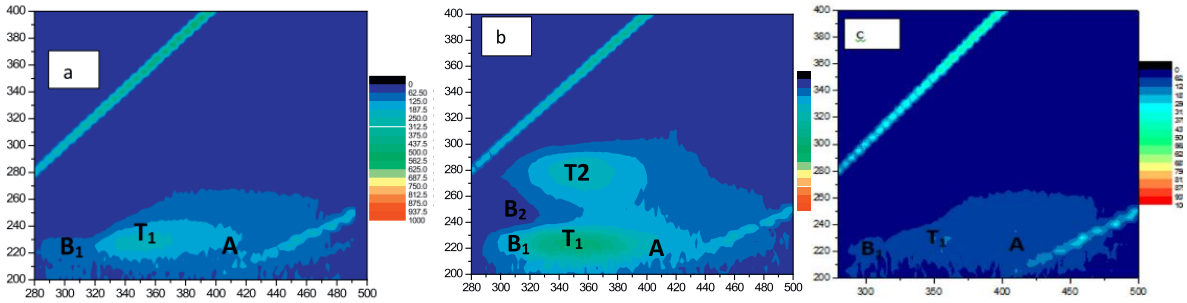



**Fig. 9 Fluorescence EEM of (a) raw water (b) Hibiscus crude seed treated water (c) Purified protein treated water Jones and Bridgeman (2017)**

### 4.5 Floc morphology and behaviour.

Over the last two decades, several techniques have been employed in floc characterisation studies, with some methods reported to be destructive to floc structures while others are non-destructive. These methods have provided a great wealth of information on floc formed by different coagulants. Jarvis *et al.* (2005a) noted that floc strength measurements are broadly classed into macroscopic and microscopic. The macroscopic methods involve measuring the energy needed for floc breakage. The microscopic also deals with the inter-particle measurement and has the advantage of close examination of floc external and internal structure. The majority of macroscopic floc studies have used variable vessel shape, volume, and impeller to study the behaviour of flocs over a range of shear forces.

Several mathematical models and imaging tools can predict floc behaviours (Moruzzi *et al.*, 2017). Most of the published tools used in floc studies are based on image and laser-based techniques which analysis floc particles sizes ranging from a few microns to millimetres. Examining flocs using the wrong technique might give rise to biased results, so selecting appropriate technology is essential (Jarvis *et al.*, 2005a).

### 4.5.1 Image-based technique

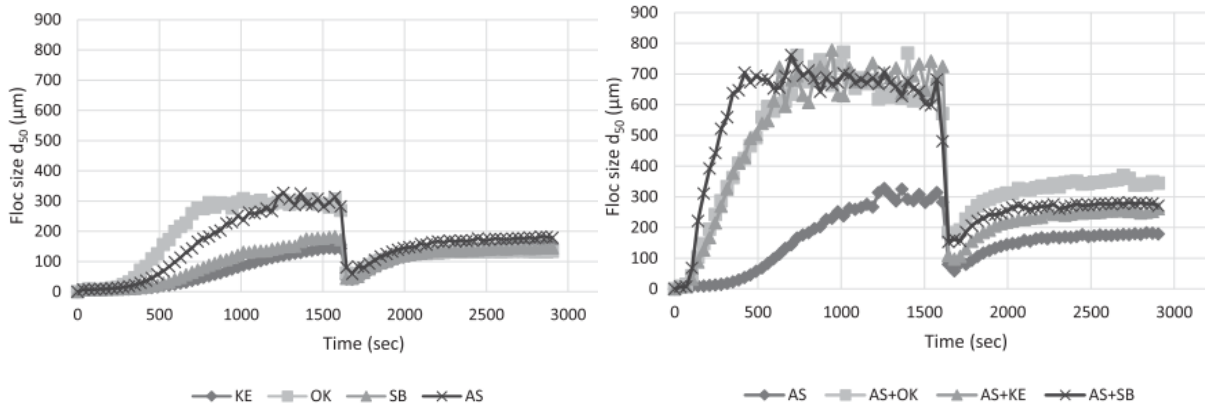
The simple microscopy technique involves manually placing samples onto the examination slides, resulting in bias due to distortion of the flocs' original structure. Unlike simple microscopy, digital imaging allows for better quality and faster enumeration of particles. Here, the flocs are usually not extracted before the evaluation, giving the advantage of structure preservation. Floc image analysis from MO and Guar Gum treated water has been achieved using a camera body coupled to a lens (Kumar *et al.*, 2016, Shen and Maa, 2016). Images derived from this method can be analysed using MATLAB image processing toolbox (Shen and Maa, 2016). Several other imaging techniques exist which have not been used in PCP floc study. Some include IN Situ SEttling Velocity which has an underwater video camera that records flocs as they settle (Shen and Maa, 2016), Online Photometric Dispersion Analyser (PDA) which has the advantage of online monitoring and good consistency of results (Moruzzi *et al.*, 2017). Another technique useful for floc examination is electron microscopy (EM), which is capable of magnification of over 10, 000 times and can show the floc structures in a detailed manner. Arguably, the sample preparatory process of advanced EM techniques like SEM (scanning electron microscopy) could damage floc particles giving rise to questionable results (Lee and Gagnon, 2016). The damage might occur during manual handling procedures such as floc transfer from the mixer, floc fixation, dying and mounting. Like digital microscopy, the EM is expensive and requires extensive training.

### 4.5.2 Laser-based techniques

The laser-based techniques measure particle size distributions by estimating variations in the intensity of light scattered as a laser beam passes through a dispersed particle. Light scattering is dependent on particle size; small and large particles scatter light at larger and smaller angles relative to the laser beam respectively. Data obtained from the angular light scatter are then used to calculate particle sizes in coagulated samples using the Mie theory of light scattering.

765 The particle size is reported as a volume equivalent sphere diameter and can be further analysed
766 for floc properties. Laser diffraction instruments used in analysing flocs generally have the
767 advantage of rapid estimation of particle sizes. These instruments include the small-angle laser
768 light scattering (SALLS) (Jarvis *et al.*, 2005a), ultra-small-angle neutron scattering (USANS)
769 technique and laser in-situ scattering and transmissometry (LISST). Their operating
770 characteristics are summarised in Table 9.

771 Equipment using SALLS, such as the Malvern particle size analyser (0.01-3500 µm, for
772 Mastersizer 3000), involves extracting a sample from suspension using a recirculating pump
773 and passing through a light transmission device for estimation of floc sizes and surface
774 characteristics. This technique might alter the floc structure and may be challenging to use with
775 low particle concentration solutions (Bridgeman *et al.*, 2008, Bridgeman *et al.*, 2010). The
776 SALLS technique has been used to study PCP coagulated water treated by Hibiscus seeds
777 (Okra, Kenaf and Roselle, Fig. 10) (Jones and Bridgeman, 2016b), with results presented on
778 their growth sizes and rates, strength, and regrowth rate at steady state before breakage, during
779 breakage and after breakage.




780

781 **Fig. 10 Growth, breakage and regrowth of Okra, Kenaf and Sabdariffa extracts and AS flocs**
782 **when used as Left: primary coagulant and Right: coagulant aid (Jones and Bridgeman, 2016b)**

783 The ultra-small-angle neutron scattering (USANS) technique (Hellsing *et al.*, 2014) uses two
784 crystal (channel-cut) to measure a monochromatic neutron beam with wavelength 2.4A and
785 accesses a range of momentum transfer between $3 \times 10^{-5}$ and $3 \times 10^{-3}$ $A^{-1}$. Hellsing *et al.* (2014)
786 investigated floc fractal properties of two Moringa species (*Moringa oleifera* and *Moringa*
787 *stenopetala*), using the ultra-small-angle neutron scattering (USANS) technique. Their results
788 indicated that *Moringa oleifera* (MO) gave a higher fractal dimension than *Moringa*
789 *stenopetala*. Consequently, MO flocs were denser and larger making them better suited for
790 treatment processes such as separation of impurities and sludge dewatering. The LISST does
791 not pump samples, reducing likelihood of flocs disruption. However, it has the disadvantages
792 of bulkiness, high costs of operation, salinity sensitivity, and might miss out very small or very
793 large flocs because of its restricted particle range (2.5-500 μm). This method has not been
794 applied in PCPs and just like others, may lead to a more thorough knowledge of the currently
795 poorly understood properties of PCPs flocs formation and breakage.

796 Other advanced floc examination methods not yet used in studying PCP flocs formation
797 mechanisms exist, including the application of computational fluid dynamics (CFD) techniques
798 through the modelling of the variation of mean velocity gradient and turbulence field in a tank,
799 and their corresponding effect on the mixing period (Bridgeman *et al.*, 2008). CFD is a
800 promising tool for this research area, but requires advanced analytical skills, and access to a
801 high-speed computing device. CFD has been used to model the flocculation process. For large-
802 scale treatment plants seeking to adopt PCPs (Sutherland *et al.*, 1994, Cornwell and Brown,
803 2017), CFD would be useful to model optimum tank configuration and CF performance.

804 Limited research conducted so far on PCPs floc morphology mainly used imaging and light
805 scattering techniques to qualify floc size, strength, breakage, regrowth and fractal dimension
806 (Table 9). Although the light scattering properties of all PCPs flocs have yet to be fully
807 understood, the present review has shown that both imaging and the light/laser scattering and

transmission techniques are good for disclosing changes in floc properties. Considering that polymers produce larger flocs, these techniques are also capable of measuring a very wide range of floc sizes (20-2 mm) (Jarvis *et al.*, 2005a), thus, making them fit for flocs monitoring. However, their high cost still limits full realisation of their potential, especially in developing climes.

Addition of polymer is generally believed in the water industry to increase floc structural characteristics such as the floc size, strength, settleability and filterability (Jarvis *et al.*, 2005a, Bratby, 2016). The limited data presented in Table 9 appears to partially support this statement in terms of floc strength and size. Jarvis *et al.* (2005b) observed a similar trend while studying a polymer-humic interaction, noting that the regrowth rate of the flocs after breakage diminished indicating poor resistance to induced shear. For the range of cationic, anionic, and non-ionic polymers evaluated in this review, the role of increasing shear on the floc strength and structural properties remains largely unknown. Going by the poor rate of regrowth recorded by Jones and Bridgeman (2016b), one could presume that increasing the shear rate caused a decline in floc strength. The decrease could be caused by involvement of the coagulating compounds in PCPs in bacterial adhesion mechanisms which reduced their overall bonding capacity. Much more research is needed in this area to adequately quantify the behaviour and effects of PCPs.

**Table 9 Tools used to study PCPs floc structural properties**

| PCPs studied | Water characteristics | Main operating conditions | Physicochemical and hydrodynamic floc characteristics | |
|---|---|---|---|---|
| Cationic starch-based flocculant, St-CTA | Synthetic water (5 types) | Mixing speed 200rpm, 50rpm with settling time 60mins. | Increase in st-CTA resulted in improvement of flocculation properties due to elevated charge attraction | (Liu *et al.*, 2017) |

| | | | | |
|---|---|---|---|---|
| | | Floc characteristics enumerated using image analysis | At lower optimal doses, larger flocs were produced for high pH values | |
| Guar Gum | Kaolin-water slurry | Image analysis: Sony Alpha NEX-5R camera body and other branded lens. Image analysed using MATLAB image processing toolbox | Salt presence improves kaolinite flocculation | (Shen and Maa, 2016) |
| MO seed protein | Synthetic water | Nano Particle Analyzer SZ-100<br><br>Contrast microscope (Q imaging) with image processing software | An inverse relationship existed between the hydraulic gradient and the floc size<br><br>Floc size at different solution pH was of the hierarchy acidic > neutral > basic | (Kumar *et al.*, 2016) |
| *Moringa stenopetala* and *Moringa oleifera* | polystyrene latices dispersed in water with $1 \times 10^{-3}$ mol $l^{-1}$ NaCl<br><br>Zeta potential: −35 mV. | BT5 USANS instrument, shear forces 200rpm, 40rpm | Proteins from two species of Moringa trees (*Moringa stenopetala* and *Moringa oleifera*) were investigated.<br><br>*Moringa stenopetala* seeds gave slightly lower fractal dimensions compared to *Moringa oleifera*<br><br>The fractal dimension of both Moringa flocculants were larger than values observed for conventional or polymeric flocculants | (Hellsing *et al.*, 2014) |
| Crude and purified extract of Okra, Sabdariffa and Kenaf as coagulant aids and coagulant | Kaolin-water slurry<br><br>Turbidity: 46±1 NTU | Malvern Mastersizer 2000<br><br>Mixing speed: 200rpm, 30rpm<br><br>Flow rate: 2L/hr | Kenaf and Sabdariffa had the lowest growth rate compared to Okra and AS. When PCPs were used as coagulant aids at steady state, floc sizes were SB: 696µm, KE: 701 µm and OK: 722 µm. AS floc size: 300 µm. | (Jones and Bridgeman, 2016b) |

| | |
|---|---|
| | Regrowth rate was poor for all crude PCPs. In contrast, AS+ Okra gave the best regrowth rate (350 µm compared to 280 and 274 µm for AS+ SB and AS+ KE).<br><br>The decreased growth rate may be attributed to nutrient leak from PCPs<br><br>Using purified Okra as aid gave highest floc size of 741 µm |

# 5 PCPs extraction and purification technique and their contribution to organic matter load in the coagulated water

Plant materials such as the seed pod, leaves and pads, barks, and flowers are primary sources of PCPs and possess adequate coagulation and flocculation properties. For most PCPs, the first processing step is harvesting the matured plant part, after which their shells, husks, and other unwanted materials are removed before further processing is done. As presented in Table 10, different plant part may require varying extraction procedure depending on the predominant coagulation compound present. The coagulant preparation is done through mechanical and chemical extraction procedures involving three stages, primary (flour preparation), secondary (oil removal and activation of coagulating compound) and tertiary (advanced compound purification), illustrated in Fig. 11 and Fig. 12. The section below presents an overview of reported techniques used in extraction and preparation of PCPs during water treatment.

## 5.1 Primary purification process

### 5.1.1 Flouring by grinding and milling

Before flouring the harvested plant such as MO and Okra, the matured and dried parts' husk is removed using an appropriate grinding technique such as mortar and pestle, grinder (Jones and

Bridgeman, 2016a) or hot/ cold hydraulic press (Camacho *et al.*, 2017). After flouring, sieving reduces the particle size (Jones and Bridgeman, 2016a). Local communities have long used this technique for water treatment because it is cheap and can be achieved without any specialised procedure (Jahn, 1981, Jahn, 1988). The flour from most PCPs can treat high turbidity water (>200NTU), however, it is ineffective in low turbidity water (<30NTU) because it adds nutrients to coagulated water which increases the DOC concentration (Table 10). Several attempts to reduce nutrient addition from the flour has been made by enclosing the flour in a muslin wrap (Pritchard *et al.*, 2010) and tea bags (Virk *et al.*, 2019). The hydraulic press method also reduces the amount of pigment and oil from the processed parts, reducing fatty substances and lipids which limits NOM and turbidity removal (Feihrmanna *et al.*, 2017). However, both the wrapping and oil-pressing only slightly reduced NOM and turbidity. The poor NOM removal performance could be because of the different solubility rates of the coagulating compounds in the PCP flour and, to overcome this challenge, some researchers have explored compound's extraction using solvents.

### 5.1.2 Water and mucilaginous extraction of coagulation compounds

Water extraction of PCP coagulating compounds improves the dissolution of polymeric compounds to enhance interaction with the dispersed particles during the CF process. It involves vigorously shaking by hand, rotary shaker or a magnetic stirrer of a given amount of the PCP flour for a given period. Water extraction is suited for PCPs with little or no oil content where the water is sufficient to extract the active coagulating compounds (Kukić *et al.*, 2015). PCPs extracted by water includes *Abelmoschus esculentus* (Okra), *Moringa oleifera* (Pramanik *et al.*, 2015, Priya *et al.*, 2017), corn and tapionic starches (Cornwell and Brown, 2017).

Aside from water extraction, fresh mucilaginous saps can be directly collected from the inner layers of plant tissues (plant paste) such as those found in some anionic and plant gums (pads

of Opuntia spp. and fresh tubers) (Lim *et al.*, 2018, Choudhary *et al.*, 2019). These mucilaginous saps have been used in treating water with both high and low turbidity. *H. rosa sinensis* leaf was noted to attain 99.08% and 17% turbidity removal in high and low turbidity water (Nidheesh *et al.*, 2017). Similarly, *Opuntia* species had an average 98% turbidity removal in water ranging 0-375NTU (Miller *et al.*, 2008). Most of the result (Table 10) from the water and mucilaginous treated samples gave an unsatisfactory NOM and turbidity removal due to incomplete dissolution of all coagulating compounds (Moreti *et al.*, 2016). Most studies employ saline extraction of compounds with a hope of better performance as is be discussed in the next section.

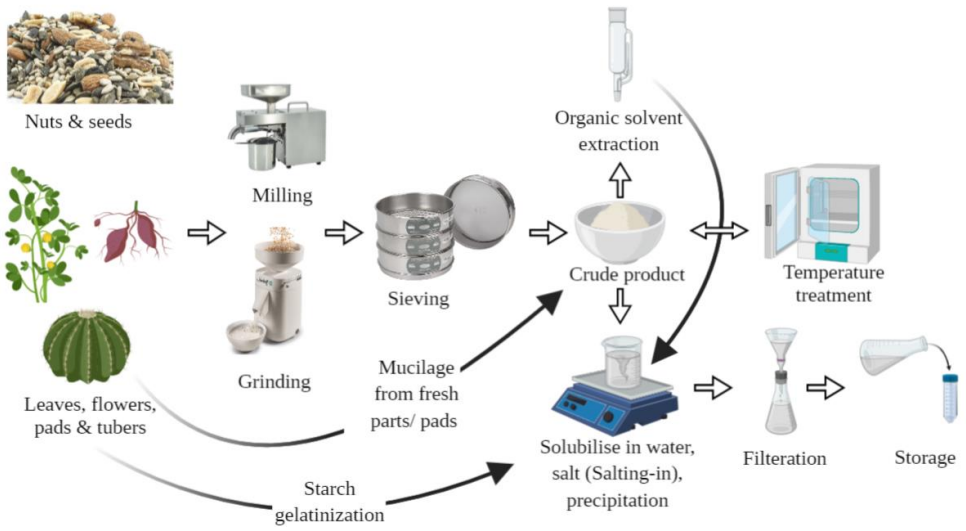



**Fig. 11 Primary and secondary PCPs extraction processes**

## 5.2 Secondary extraction process

### 5.2.1 Salt extraction

Some of the coagulating compounds dissolve faster in saline solutions and have better NOM and turbidity removal potential when prepared is this way due to the solution's increased ionic strength (Noor *et al.*, 2015). The salting-in process increases the compound’s efficiency more than the salting-out process, explained by DebyeHuckel theory and John Gamble Kirkwood's

884 principle, respectively. A lower ionic strength solution generally improves solutes' solubility,
885 including protein present in the plant material (Baldwin, 1996). Higher ionic strength (salting-
886 out) decreases protein solubility and might dehydrate the protein (Baldwin, 1996), whereas
887 neutral salts precipitate carbohydrates. These processes are complex and can be affected by
888 temperature change, pH, compounds, and salt concentration.

889 Salt type arguably has no known effect on Moringa seeds' CF performance (Madrona *et al.*,
890 2012, Megersa *et al.*, 2019). However, one would expect that the different salts would
891 differentially influence performance. Sodium chloride (NaCl) extracted PCPs had higher
892 turbidity removal than potassium chloride (KCl) salt (Mageshkumar and Karthikeyan, 2016).
893 The difference in performance was because of higher solubility rate and hydration energy of
894 NaCl (NaCl: -406 and -363 kJ/mol while KCl: -322 and -363 kJ/mol respectively). Compared
895 to the $K^+$, the water molecules favourably solvates the $Na^+$ ion in water (Patil *et al.*, 2015).
896 Further study on other PCPs would be useful to know if different salt types influence their
897 NOM performance.

898 Improved NOM removal by MO salt extract is caused by breaking down its protein-protein
899 bonds (Ndabigengesere *et al.*, 1995). From Table 10, the salting-in process generally reported
900 higher NOM and turbidity removal than the water extracts. Other saline products with
901 established NOM removal abilities include isolated seed protein of *Strychnos potatorum*
902 (Nirmala) (Arunkumar *et al.*, 2018), acorn fruit (Antov *et al.*, 2018), kenaf, russel and okra
903 saline products (Jones and Bridgeman, 2016a, Okoro *et al.*, 2021).

904 It is clear from the result that the salt extraction process gives better treatment benefit than the
905 flouring and water extraction methods. Despite this advantage, their coagulated water still has
906 nutrient addition problems and high DOC, giving treated water with turbidity above the

maximum contaminant limit (MCL) of both WHO and US EPA, which have led to further examination of new products derived from other experimental techniques.

### 5.2.2 Organic solvent extraction

The main aim of PCP extraction using organic solvents is to exclude lipids and non-coagulating compounds from the PCPs. The delipidation process reduces nutrient infiltration into treated water which is a major hindrance to its NOM and turbidity removal performance and PCPs commercialisation. Several other organic solvents, such as hexane, have been used to defat crude plant parts through gradual stirring or a Soxhlet apparatus (Carvalho Bongiovani *et al.*, 2014, Feihrmanna *et al.*, 2017, Okoro *et al.*, 2021). Several reports have noted improvement in NOM and turbidity removal after extraction MO protein with ethanol (Amante *et al.*, 2015) and hexane (Carvalho Bongiovani *et al.*, 2014, Okoro *et al.*, 2021). Antov *et al.* (2010) revealed 16 times reduction of crude extract organic matter concentration after purification. Despite the improvements recorded, PCPs purified using this method still increase the treated water DOC. Combining these PCPs might solve this DOC problem, so, more studies should explore their combined performance with CCPs such as alum.

## 5.3 Tertiary extraction processes

A significant milestone in CF water treatment using PCPs is developing techniques for isolating and storing coagulating compounds. Separation of the suitable coagulating compounds in the aqueous or solvent extracted products is based on their difference in solubility (e.g. ammonium sulphate precipitation) (Arunkumar *et al.*, 2018), affinity to the ion column or exchanger (ion-exchange chromatography) (Ghebremichael *et al.*, 2005, Bodlund *et al.*, 2014, Jones and Bridgeman, 2015), and size of molecular masses, which ranges between 5-250 kDa (SDS-PAGE) (Jones and Bridgeman, 2015, Taiwo *et al.*, 2020).

Most of these separation process starts with the precipitation technique using inorganic and highly soluble salt to alter the compound's (mostly protein) solubility level using a high salt concentration such as ammonium sulphate. Introducing a high salt concentration (salting-out process) competes with the protein molecules for available water molecules, leading to the protein's precipitation (Wingfield, 1998). The precipitation process occurs in stages with the precipitates recovered at each stage by centrifugation before further purification steps. The precipitation process's efficiency depends on the mW of the protein, pH, and temperature of the solution (which could affect enzyme activity), and the type of polar groups present. Dialysis removes salt from the protein through the selective exclusion of protein using a membrane barrier after which the dialysed protein is chromatographed and stored for use. A summary of some of the tertiary purification techniques are presented in Table 10.

Protein fractions from MO have successfully been isolated using these processes (Ndabigengesere *et al.*, 1995, Baptista *et al.*, 2017). Also, 0.73mg/l dosage of purified protein from Common Oak (*Quercus robur*) gave a treatment efficiency of approximately 72.3% against 15.84% of its crude product (Antov *et al.*, 2010). Compared to crude products of Okra, Sabdariffa and Kenaf, which increased DOC of treated water by 65%, 61% and 55%, their purified products reduced DOC concentration (Jones and Bridgeman, 2016b). Similarly, purified protein from *Vigna unguiculata* and *Parkinsonia aculeata* performed 5-6 times better than their crude products (Marobhe *et al.*, 2007).

Compared to other extraction methods, these techniques provide water quality that in some cases satisfies the MCL (Ndabigengesere *et al.*, 1995, Baptista *et al.*, 2017, Camacho *et al.*, 2017). The major challenge of using these processes is their cost since they involve many purification processes that would be challenging to implement in developing and poor communities. Also, their treated water contains lower level of DOM making them potential DBP precursors, especially in a chlorinated system.

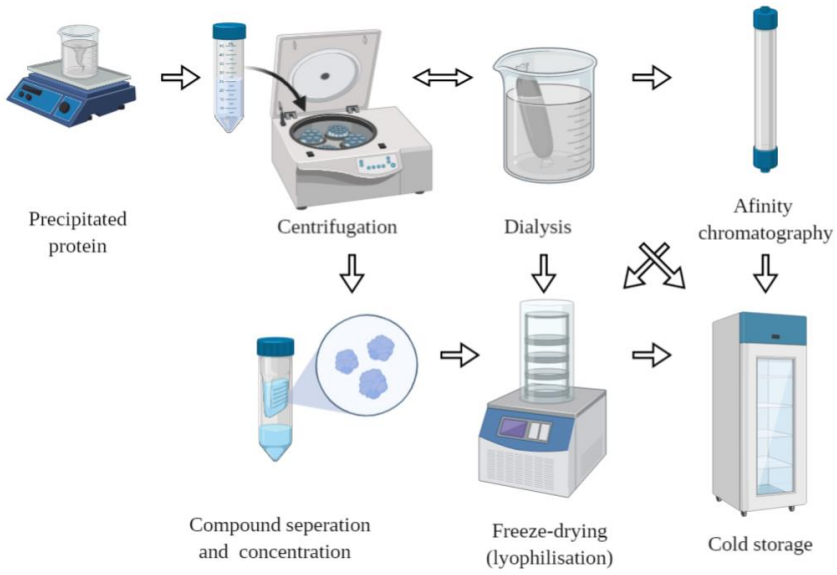



**Fig. 12 Tertiary (advanced) PCPs extraction processes**

## 5.4 PCPs Storage

Storage of PCPs after preparation is equally important, especially since the coagulating compounds easily denature. The most accessible storage form for local communities is storing the whole seed, ground flour or water extracts (Jahn, 1981, Jahn, 1988). Their use is mainly due to ease of handling and storage since users need not worry about acquiring purification material and expensive storage. The advanced purification and storage techniques such as delipidation and purification require cold storage conditions and so may be unsustainable for the poor and rural.

PCPs have different lifespan and storage requirements due to their biodegradable nutrient content, which varies depending on purification undergone. A study using MO flour, reported that storing for 1-5 months duration at both room (28$^{o}$C) and cold storage (3$^{o}$C) condition continuously resulted in declining performance suggesting that, irrespective of temperature, coagulating protein degrades with time (Katayon *et al.*, 2006). Other findings on PCP storage include: that purified MO and its saline extract can be stored at 25$^{o}$C and -18 $^{o}$C respectively

971 (Garcia-Fayos *et al.*, 2016), while stored Okra, kenaf and roselle retained approximately 70%
972 of their turbidity removal performance after 10 days (Jones and Bridgeman, 2016a).

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975 **Table 10 Water treatment performances of selected PCPs**

| PCP | Water Sample | Operating Characteristics | Optimum Dose | Treatment recorded with implications | Ref. |
|---|---|---|---|---|---|
| *Moringa Oleifera*-MO (drumstick) | Stream water, Malaysia | ζ-potential of raw water ranged 21.4mV to -27.6mV. pH 6.9 to 7.5. Combined partial neutralisation and micro bridging.<br>ζ-potential of treated drop within range -18.8mV to – 22.9mV<br>Purification: F-D[4] | Variable | Different MO-Alum dosage matrixes used<br>MO was efficient in low turbidity removal due to 83% oil removal.<br>*Implications*: using MO as primary coagulant reduced turbidity below WHO recommended guideline (5NTU). | (Muyibi and Alfugara, 2010) |
| *Moringa Oleifera*-MO (drumstick) | Pirapo River, Brazil | Mechanism of coagulation was adsorption and charge neutralisation<br>Purification: F-D[4, 5]-SE | Optimum dose was 30mg/L. | -$Sv_{he}$ gave best removal efficiency for turbidity (95%) and $UV_{254}$ (83%).<br>0.1mg-0.4mg/L of anionic polymer as flocculant aid achieved settlement in 5min compared to 1hr using $Sv_{he}$<br>*Implications*<br>Defatting PCPs reduces organic matter content in seed | (Carvalho Bongiovani *et al.*, 2014) |
| *Moringa Oleifera*-MO (drumstick) | Reservoir water | ºC of water: 22 ± 2, Turbidity: 2.60 ± 0.15NTU. mW fractions of raw water were between 1kDa-30kDa.<br>300rpm.<br>CF experiment conducted using Jar tester under mixing rate 300rpm for 5min then 40rpm for 20mins. Sedimentation time was 1hr. | 70mg/L | -DOC removal: 56% for BAC, BAF 51%, SF 45%, alum 27% and MO 22%.<br>In water sample mW distribution of HAAFP, THMFP and NDMAFP were of order 52% of 1 kDa, 22% of 1-3 kDa, 14% of 3-10 kDa, 7% of 10-30 kDa and 5% of >30 kDa.<br>-N-nitrosodimethylamine (NDAFP) reduction ranged 85%-98%; THMFP and HAAFP treatment efficiency followed the order MO < Alum < SF < BAF < BAC | (Pramanik *et al.*, 2015) |

| | | | | | |
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| | | Dosage range: 50-100mg/L<br>Purification: F-WE | | *Implications*<br>The major contributor to THMFP, HAAFP and NDAFP was organic matter fraction <1 kDa consisting mostly of the aromatic and carboxylic group.<br>-Alum performance exceeded MO-PCP$^{CE}$. | |
| *Moringa Oleifera*-MO (drumstick) | River water, Pirapo, Brazil | Turbidity 50-200NTU.<br>Studied 1% and 5% MO concentration<br>CF test conducted using Jar tester. Fast mix rate was 100rpm for 3min; low mix rate was 15rpm for 15mins.<br>Dosage range: 3-75mg/L<br>Purification: F-D-WE-PE[4,7,8,9] | Globunin: 13mg/L, Albumin 13.78mg/L | -MO main composition noted as Globulins (53%) and Albumins (44%).<br>-87.40% colour removal, 89.71% turbidity, 79.46% $UV_{254}$. Good DOM reduction potentials<br>*Implications*<br>-PE PCP slightly contributed to DOM level of water although level better than MO1% and MO5% | (Baptista *et al.*, 2017) |
| *Moringa Oleifera*-MO (drumstick) | Soil water and *Microcystis aeruginosa* simulated water | Turbidity: low and high turbidity surface water (5, 10, 30 and 60NTU). Mixing rate: 743 $s^{-1}$ (200 rpm) for 2 min and a slow mixing gradient of 24 $s^{-1}$ (20 rpm) for 15 min.<br>Settling time range: 10-120 min<br>1L Jar Tester with 4 paddles.<br>Dosage range: 0, 50 and 100mg/L.<br>Purification: F-D[2,3,4]-SE | 50mg/L[4] achieved 90% chlorophyll removal | -Turbidity removal of ≥ 85%. Low turbidity water removal efficiency was 60%; All coagulants removed 40-50% organic matter water content. pH of MO-PCPs does not influence water pH<br>*Implications*<br>-All MO-PCPs added to DOC of water.<br>-Should be combined with other treatments for better efficiency | (Camacho *et al.*, 2017) |

| | | | | | |
|---|---|---|---|---|---|
| *Moringa Oleifera*-MO (drumstick) and *Cyamopsis tetragonoloba* | Kaolin-water slurry | CF experiment conducted with Jar tester with agitation speed: 50 rpm, zeta potential: -30mV<br>Dosage range: ~10mg/L<br>Purification: F-WE | pH: 8.0 | For both, CF mechanism was by charge neutralisation, sweep coagulation and adsorption-bridging. *C tetragonoloba* improved performance attributed to the bridging effect as reflected in SEM.<br>-MO improved performance of Alum; MO treated water consumed more chlorine. Alum-MO combined coagulant reduced SUVA value by 68% while MO by 67%<br>For *Cyamopsis tetragonoloba*, Enhanced coagulation achieved pronounced removal of DOM, $UV_{254}$ absorbing materials and reduced chlorine consumption. $SUVA_{254}$ for C. tetragonoloba and Alum/ C. tetragonoloba value was reduced by 50.7% and 40.09%<br>*Implications*<br>Cyamopsis tetragonoloba-Alum produced bigger flocs sizes than MO-Alum coagulant likely due to structural variations.<br>-Using alum-MO, maximum floc size was 315nm (92% particle), alum was 193nm (81%) and C. tetragonoloba gave 246nm.<br>- DOM level increased in water after treatment. | (Priya *et al.*, 2017) |
| *Moringa Oleifera*-MO (drumstick) | NOM and cyanobacteria simulated waters | Low (11.6NTU) and medium (61.7NTU) turbidity water.<br>Dosage range: 50 mg/L<br>Purification: F-$SE^3$ | 50mg/L | -Compared to the performance of Alum reported by previous research MO performed satisfactorily with turbidity and chlorophyll a: 80%, DOC 70-80%, 80- 90% for $UV_{254}$.<br>-After MO CF, high DOC adsorbed by PAC correlated with high adsorbent doses | (Teixeira *et al.*, 2017) |

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| | | | | *Implications:* MO addition could not remove all DOC of water. However, combination treatment (DAF-PAC) significantly reduced concentration. | |
| *Abelmoschus esculentus*- Okra | Bourn brook river water, UK. | Fluorescence EEM analysis was conducted using Varian Cary Eclipse Spectrophotometer. Excitation: 200-400nm, 5nm; emission: 280-500nm, 2nm with slit: 5nm. Raman value (exc: 348nm, emm. 395nm)<br>Dosage range: 0-200mg/L<br>Purification: F-SE-TT[1] | 40mg/L (TT) and 60mg/L (CE) | Turbidity in CE: 84% while that of TT was 92.33%. About 99% turbidity removal at pH 4.0.<br>-pH of the water sample was not affected after treatment with PCP[TT, CE]<br>*Implications:* fluorescence spectroscopy application in the study of DOM content of PCPs<br>-DOC increase after treatment recorded. | (Jones and Bridgeman, 2015) |
| *Abelmoschus esculentus*- Okra | Kaolin-water slurry | Initial water turbidity 100-200 NTU<br>CF test conducted using Jar tester. Mixing rate was 200 rpm (G=240 $s^{-1}$) for 1 min then 30 rpm (G=23 $s^{-1}$) for 30 min.<br>Dosage range: 40-60mg/L<br>Purification: F[1] -SE[3] | 80mg/L | -Highest removal Okra salt PCP: 93%, Okra salt PCP[TT]: > 97%<br>-About 99% turbidity removal at pH 4.0<br>*Implications*<br>- Performance of denatured okra seed surpassed crude extract<br>- PCP more effective in high turbidity water due to reduced organic matter content | (Jones and Bridgeman, 2016) |
| *H. rosa sinensis* leaf | Kaolin-water slurry | Low and high turbidity water were 60NTU and 325NTU, respectively. Mixing rate were 160rpm for 1mins; 40rpm for 20mins. Coagulation mechanism reported was sweep floc<br>Dosage range: 0-15mg/L | 6mg/L (low turb.) and 1.5mg/L (high turb.) | Poor turbidity removal in low turbidity water. Evidence of DOC addition. Could be a good coagulant aid to CCPs.<br>*Implications:* higher dose of extract increased turbidity concentration in water. Salinity increased solid concentration in water. | (Nidheesh *et al.*, 2017) |

| | | | | | |
|---|---|---|---|---|---|
| | | Purification: $F^6$ -WE | | | |
| Opuntia focus-indica | Kaolin-water slurry | ζ-potential of water ranged – 7mV (pH 3.0) to – 37mV (pH 12.0)<br>Adsorption with bridging mechanism prevalent<br>Dosage range: 0-100 mg/L<br>Purification: F-WE | pH: 10.0; dose: 35mg/L | -pH increases with decreased ζ-potential<br>-Turbidity @ pH <5.0: 40%; @ pH 6.0>x<10.0: 92%; @ pH 10.0>x<12.0: 78%<br>-Floc size @ pH <5.0: 10-30µm; @ pH 6.0>x<10.0: <10 µm; @ pH 10.0>x<12.0: 70-400 µm<br>*Implications:* at optimum performance, more organic matter was added to water<br>-Floc morphology and characterisation information required to understand the nature of flocs formed. | (Bouaouine *et al.*, 2018) |
| Strychnos potatorum | Kaolin-water slurry | Initial turbidity 200-300NTU. Mixing speed 200rpm ($380s^{-1}$)<br>Dosage range: 0-2.5mg/L<br>Purification: F-D-PE | 10µl/ml | -Best turbidity removal was 84%. Purified protein performance was 5-10 times higher than water extract.<br>*Implications:* coagulation activity reported for a 10µl/ml dose of coagulant was 84% with a possible addition of DOM to water. | (Arunkumar *et al.*, 2018) |
| *Margaritarea discoidea*<br>Fruit Seed Extract<br>FSE | Kaolin-water slurry | Turbidity 175NTU<br>Coagulation mechanism was likely adsorption and bridging linked to long chain polymeric structure and galactose unit of FSE<br>Dosage range: 2.5-25ml/L<br>Purification: $F^6$ | 10ml/L | >90% solids removed. Colour addition after treatment. Micro and macro floc, and faster sedimentation rate recorded.<br>*Implications:* more study required to access impact of PCPs on treated water DOC. | (Oladoja *et al.*, 2017) |

| | | | | | |
|---|---|---|---|---|---|
| *Phaseolus vulgaris* and *Strychnos potatorum* | Synthetic water | Turbidity was 100NTU and 500NTU. Mixing rate was 100rpm and 40rpm for 4min and 25min, respectively.<br>Purification: F-WE[10] | 250-1000mg/L | -Turbidity removal efficiency was 87.3% for 500NTU water and 76.1 for 100NTU water<br>-Turbidity removal efficiency was 90.6% for high turbidity water and 84.2% for low turbidity water<br>*Implications*: NaCl elution solvent recommended over water and NaOH; Ultrafiltered products derived from water extraction process gave lower residual organic load compared to other products | (Muthuraman and Sasikala, 2014); 3,4--oladoja |

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# 6 Improved NOM removal in PCP coagulated water

## 6.1 Engrafting Technique

Some shortcomings of the PCPs such as low shelf life and poor NOM removal caused by leaching of nutrients have been resolved through a graft polymerization process. In this process, the monomeric molecules that produce the polymers basic unit are covalently bonded and polymerized as side chains onto the backbone (the main polymer chain) (Sherazi, 2014). This process imparts different functional groups to a polymer resulting in a new advanced material with better NOM removal abilities (Chua *et al.*, 2020). A variety of grafting processes exist, such as conventional redox grafting using chemical, ultraviolet (UV) light irradiation, γ-ray irradiation, electron beam, and microwave irradiation. The microwave irradiation is the most reported due to its simplicity, and the ability to allows selective heating of materials. It can also solely generate free radicals resulting in a higher grafting percentage (Chua *et al.*, 2020).

These hybridization processes have been applied to natural polymers, including polysaccharides. Cationic starch has been engrafted in 2,3-epoxypropyl trimethyl ammonium chloride (GTA) (Lin *et al.*, 2013). The engrafted coagulant improved NOM removal and resulted in full floc recovery by the end of the fourth mix regime. Grafting of coagulants can improve floc characteristics and strength for optimum NOM removal. So, more studies are encouraged using grafting techniques to improve NOM removal by PCPs.

## 6.2 Combination treatment using PCPs and CCPs

The blending of chemical coagulants with the PCPs gives advantages of improved NOM removal (Carvalho Bongiovani *et al.*, 2014), better sludge degradation, longer coagulant shelf life, reduced CCP dosage (Muyibi and Alfugara, 2010) and paves the way for commercialization of the coagulants. Combining coagulants can overcome their performance

limitation and lead to improved NOM removal due to the combined action of two or more of the CF mechanisms.

Combination of alum and *C. tetragonoloba* (a polysaccharide) reduced aromatic fraction of NOM due to the cumulative effect of sweep flocculation and particle bridging (Priya *et al.*, 2017). Combining Hibiscus plant seeds, i.e., Roselle, Kenaf and Okra, with alum resulted in improved growth sizes of 696 µm, 701 µm and 722 µm. Also, their floc regrowth after breakage increased. The use of MO seed reduced alum dosage by 40% (Muyibi and Alfugara, 2010). MO has also been combined with TANAC anionic polymer (PA 0823), resulting in decreased sedimentation time from 60 min to 15 min (Carvalho Bongiovani *et al.*, 2014). Other combination study includes autoclaved rice starch (a weakly anionic PCP) and aluminium salts (Choy *et al.*, 2016).

PCP hybridization or combination treatment produces a smaller volume of sludge, which is easier to dewater and thicken than those produced by the CCPs such as alum. Low sludge volume could also reduce the bulkiness of treatment facilities and perhaps lead to the elimination of thickening (Cornwell and Brown, 2017). Combination treatment results in lower metals content in sludge which implies that discharge would more easily comply with regulatory limits, and more discharge and reuse options would be available.

## 7 Addition of nutrients and dissolved organic matters (DOM) by PCPs

Most of the coagulating compounds present in PCPs are similar to those reported as DBP precursors during disinfection water treatment. Literature reports that amino acids, such as tryptophan and tyrosine (Fig. 13), can form THMs and HAA (Hong *et al.*, 2009). Asparagine and aspartic acid are also HAA precursors (Chu *et al.*, 2017). Aliphatic compounds present in PCPs, including carbohydrate and fatty acids, are also established precursors to brominated disinfection by-products which are apparently more cytotoxic and genotoxic than the

chlorinated species. Some iodinated by-products precursors are alicyclic molecules, rich in carboxylic acid, phenolic and carboxylated compounds (Hao *et al.*, 2017). Tea and coffee, derived from plant materials, contain caffeine, d (-) quinic acid, d (+) galactose, catechol, chlorogenic acid, (+) catechin hydrate, epigallocatechin gallate and gallic acid (Bond *et al.*, 2016). Spiking these compounds with chlorine produced chloroform levels that were generally higher (47.6 ± 0.3%) than that of typical NOM surrogates.

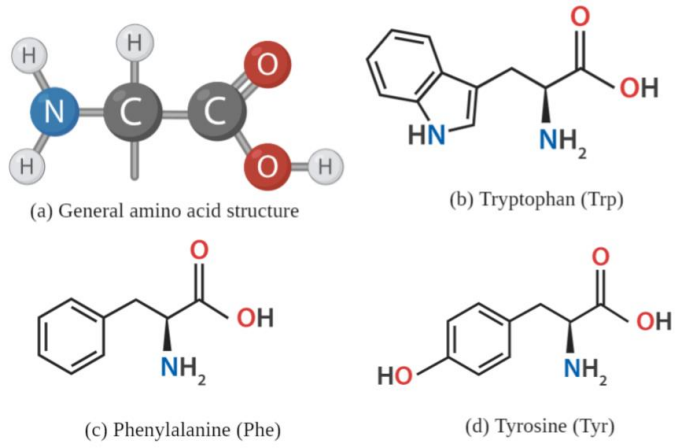


**Fig. 13 (a) Selected molecular structures showing (a) amino acid, (b) tryptophan, (c) phenylalanine, (d) tyrosine**

Approximately 700 DBPs have been discovered, and of these DBPs, the US EPA regulates only 11, i.e., $THM_4$, $HAA_5$, bromate and chlorite (Richardson and Ternes, 2018). Several other DBPs exists including 5 nitrosamines (N-nitrosodimethylamine (NDMA), N-nitrosodipropylamine (NDPA), N-nitrosodiphenylamine (NDPhA), N-nitrosodiethylamine (NDEA), and N-nitrosopyrrolidine (NPYR); chlorate; and 2 aldehydes (formaldehyde and acetaldehyde) (Richardson and Ternes, 2018). Recent evidence from epidemiological studies have shown that long-term consumption of drinking water containing nano and micro-level concentration of these DBPs, could result to health challenges like colorectal and bladder cancer, and defects during birth (Matsuhiro *et al.*, 2006, Richardson and Kimura, 2017).

Unfortunately, most of the rural HWTS systems, including the PCPs, have few regulatory compliance checks to determine the disinfection process's impact. Absence of literature on DBP formation potential of these PCPs implies that suggestions of their non-toxic attributes may be questionable. So, comprehensive research to appraise the contribution of leached nutrients from these PCPs to DBPs would be beneficial both for HWTS and large-scale treatment considering their use as a coagulation aid. PCP coagulated water deteriorates with time and other operating factors due to high nutrient (DOM) presence that can potentially increase nitrogenous DBP concentration, rendering water unsafe (Richardson and Kimura, 2017). These factors, including temperature, pH, coagulant dosage, and contact time, should be evaluated to establish their role in DBPs formation in PCP coagulated water.

## 8 Future research

In the future, the main challenges for PCP research lie in four key areas (i) Viability and practicality (ii) residual nutrients from PCPs to avoid disinfection by-products (iii) NOM and flocs analysis and (iv) Field studies involving properties optimization to adapt to different operating conditions. To reduce some of the current issues in the use of PCPs as previously illustrated in Table 4, a few studies combined PCPs with conventional coagulants and have shown promising performance. Besides, incorporating PCPs potentially could reduce the carbon footprint of the overall treatment process since they are of plant origin and are biodegradable materials. Hence, more studies should be conducted to close this gap in the literature. In more detail, the main focus of PCP studies in the future should be as follows:

a) Viability and practicality of real-world implementation and sustainability: For medium- to large-scale water treatment, large-scale production of these PCPs is required, which may not be cost effective and sustainable as they require larger climate-controlled storages spaces, and all-year supply. Most of the PCPs are used for food or are cash

crops. The additional demand that would be placed by water treatment use on these crops would impact on their accessibility globally. This problem can be addressed by focusing on other non-food/ - non-cash crops with potential for water treatment. Also, a comparative study on cost of using PCPs for full-time use by small, medium, and large water systems is necessarily.

b) Residual nutrient from PCPs: nutrient addition by the PCPs can impart odours and colour to drinking water. This problem can be reduced by using purified coagulation products of most PCPs, minimising odours and colour control. The presence of similar compounds in PCPs that are established precursors to DBPs formation means that these PCPs are potential precursors to forming both carbonaceous and nitrogenous DBPs in chlorinated systems. These DBPs and emerging contaminants in water are now a major concern in most water treatment systems due to their health effects. Most of their potential DBPs formation ability has not been evaluated. Therefore, research into the unintended consequences of using these PCPs with a disinfection process should be examined for future research needs. The result will also establish whether their current use with a disinfection process such as chlorination, results in negative or positive consequences.

c) NOM and flocs analysis: both the efficacy and process of NOM removal by most PCPs is still unclear and underreported due to poor access and use of analytical tools and techniques. Likewise, their floc morphology has scarcely been reported, which has hindered their full comprehension and use in water treatment. NOM removal by other potential PCPs should be conducted using the available analytical methods in order to identify potentially more efficient PCPs.

d) Field studies involving properties optimization to adapt to different operating conditions: most of the research to-date has presented findings using kaolin (model)

water samples, which may not represent the typical quality in natural water sources. Therefore, more water types and sources should be used for further studies to establish the performance of PCPs under variable operational factors. These studies should consider seasonal influence on polymer dosages and associated performances, the impact of coagulation pH, alkalinity, temperature, and storage duration.

## 9 Concluding remark

Most of the plant-based coagulants (PCPs) evaluated in this review showed some remarkable turbidity and NOM removal results and can therefore be used in underdeveloped rural community where there is an absence of any other coagulant source or may be used for other non-potable purposes. Also, their purified forms can remove turbidity and produce WHO acceptable drinking water. However, if the treatment objective is mainly to remove NOM, then the PCPs would require further purification or structural improvement.

As captured in the review, NOM removal by PCPs is underreported due to inadequate understanding and application of characterisation techniques and limited access to tools. More research involving the current NOM and PCPs characterisation tools and techniques would improve awareness and assist researchers and operators in optimising PCP's performance. All PCPs contribute nutrients to the treated water, thus increasing the risk of disinfection by-product formation. More research effort is required on the contribution of leached nutrients from PCPs and the DBPs formation potential. Further, hybridized PCPs, which have enhanced turbidity removal ability, should be further researched for their NOM removal and DBP formation potential to provide useful information on their performance. This review creates awareness of current tools and techniques used in characterising PCPs biophysical properties, performance, and NOM removal. The review will improve understanding of PCPs suitability for water rejuvenation under varying conditions, and motivate water managers, researchers, and decision-makers, to deploy them at full-scale.

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**CRediT authorship contribution statement**

Benjamin U. Okoro: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing - original draft. Soroosh Sharifi: Conceptualization, Supervision, Writing - review & editing. Mike Jesson: Conceptualization, Supervision, Writing - review & editing. John Bridgeman: Conceptualization, Writing - review & editing.

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