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1 **Investigating the characteristic strength of flocs formed from crude and**
2 **purified Hibiscus extracts in water treatment**

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11 **Abstract**

12 The growth, breakage and re-growth of flocs formed using crude and purified seed extracts of
13 Okra (OK), Sabdariffa (SB) and Kenaf (KE) as coagulants and coagulant aids was assessed.
14 The results showed floc size increased from 300 μ m when aluminium sulphate (AS) was used
15 as a coagulant to between 696 μ m and 722 μ m with the addition of 50mg/l of OK, KE and SB
16 crude samples as coagulant aids. Similarly, an increase in floc size was observed when each
17 of the purified proteins was used as coagulant aid at doses of between 0.123 and 0.74mg/l.
18 The largest floc sizes of 741 μ m, 460 μ m and 571 μ m were obtained with a 0.123mg/l dose of
19 purified Okra protein (POP), purified Sabdariffa (PSP) and purified Kenaf (PKP)
20 respectively. Further coagulant aid addition from 0.123 to 0.74mg/l resulted in a decrease in
21 floc size and strength in POP and PSP. However, an increase in floc strength and reduced d₅₀
22 size was observed in PKP at a dose of 0.74mg/l. Flocs produced when using purified and

23 crude extract samples as coagulant aids exhibited high recovery factors and strength.
24 However, flocs exhibited greater recovery post-breakage when the extracts were used as a
25 primary coagulant. It was observed that the combination of purified proteins and AS
26 improved floc size, strength and recovery factors. Therefore, the applications of Hibiscus
27 seeds in either crude or purified form increases floc growth, strength, recoverability and can
28 also reduce the cost associated with the import of AS in developing countries.

29 **Keywords:** Hibiscus extracts, floc strength, coagulants, purified proteins, water treatment

30

31 **1.0 Introduction**

32 For decades, different chemicals have been applied in water treatment to aid the removal of
33 contaminants and harmful substances. Chemical coagulants are added to destabilise the
34 dispersed colloids, with charge neutralisation, adsorption and sweep flocculation being the
35 major mechanisms of action (Duan and Gregory, 2003). Accelerated sedimentation is
36 achieved by aggregating the flocs via slow mixing (flocculation) to form larger macro flocs
37 facilitating removal in a sedimentation tank. However, to achieve satisfactory treatment, flocs
38 must demonstrate sufficient strength so as not to be broken by the turbulent flow field found
39 in the flocculator and clarifier. Thus, the merit of each coagulant is judged based on, *inter*
40 *alia*, the strength, size and density of the flocs formed. Previous work has observed that
41 smaller flocs are more likely to resist rupture than larger flocs but may pose some challenges
42 during removal compared to bigger flocs (Boller and Blaser, 1998, Jarvis et al., 2005c), as the
43 mechanism and general mode of floc transportation is hampered if the flocs are small in size
44 and so cannot settle effectively. Conversely, it can be argued that smaller and more compact
45 flocs with tighter bonds will resist breakage and settle faster than larger, weaker flocs (Jarvis
46 et al., 2005c). However, it has been reported that the stronger the flocs, the larger they can

47 grow under certain shear conditions (Mühle, 1993). However, (Sharp et al., 2006a) revealed
48 that larger flocs can easily break in high turbulent condition, because they are weaker. It can
49 be deduced here that highly compact flocs are generally stronger and smaller in size. Thus, it
50 is challenging to prevent floc breakage under normal plant conditions, particularly in highly
51 turbulent areas; consequently, the regrowth potential of flocs post-rupture is of interest.

52 Many researchers have investigated floc properties, including floc strength, using different
53 coagulants and under different plant operating conditions. Previous work has monitored floc
54 growth, breakage and re-growth phases after the introduction of high shear rate (Jarvis et al.,
55 2005b, Yu et al., 2012, Xu et al., 2014). Yukselen and Gregory (2004) and Li et al. (2007)
56 observed in their separate studies that AS flocs exhibit irreversible breakage. Conversely, Yu
57 et al. (2014) evaluated the property of kaolin-alum flocs at low pH and showed that 100%
58 floc recovery is possible if AS or Kegging polymer $Al_3 [AlO_4Al_2(OH)_{24}(H_2O)_{12}]^{7+}$ was
59 used as coagulant at acidic pH. Several others workers have also reported the importance of
60 low pH in improving floc strength and recoverability using different chemical coagulants
61 (Cao et al., 2010, Sun et al., 2011). Sharp et al. (2006b) investigated the properties of ferric-
62 NOM flocs and revealed that flocs generated by iron salts are larger and more resistant to
63 breakage than AS flocs, resulting in accelerated settling. Beside AS producing irreversible,
64 smaller and weaker flocs (Yukselen and Gregory, 2004, Sharp et al., 2006b, Li et al., 2007),
65 research has also been undertaken to investigate the relationship between residual aluminium
66 in water and Alzheimers disease (Gauthier et al., 2000, Flaten, 2001). Cost issues associated
67 with the import of coagulants such as AS exacerbate these issues further for developing
68 countries. It is, therefore, imperative to search for alternative natural coagulants and
69 coagulant aids that will lower the cost of water treatment in developing countries and also
70 improve water treatment efficiency. By so doing, the number of deaths resulting from

71 drinking contaminated water supply could be lowered in rural areas and life expectancy
72 increased.

73 Recently, several natural materials have been studied to assess their coagulation potential in
74 water treatment. Preliminary investigation of some of these natural extracts has so far
75 provided encouraging results for people in developing countries. Naturally-occurring plant
76 extracts including *Moringa oleifera* (MO), *Cactus latifaria*, and *Mustard seeds*, have
77 coagulation capability and can be used in water treatment (Jahn Samia, 1998, Diaz, 1999,
78 Bodlund et al., 2014). Similarly, other natural plants, such as Hibiscus, are widely used in
79 many tropical countries because of their nutritional values. Among the many Hibiscus plant
80 species, only OK seed pod has been investigated as a flocculant in the treatment of water and
81 wastewater (Agarwal et al., 2001, de Jesus et al., 2013). Recently, Jones and Bridgeman
82 (2016) have demonstrated the capability of OK seed extract in removing turbidity and bacteria
83 in river water. Additionally, it has been reported that activated carbon derived from KE fibre,
84 another Hibiscus plant could be used to treat water and wastewater with high heavy metal
85 contents (Chowdhury et al., 2012). Conversely, there is no known report on the use of SB
86 seed in either water or wastewater treatment. However, SB extract was found as an effective
87 inhibitor of microbial growth when it was applied on some isolated microbes (Nwaiwu et al.,
88 2012). Most of the reported work has centred on the coagulation activities of the extracts,
89 whereas problems related to floc strength and recovery have not been investigated, despite
90 their importance in the treatment process. Therefore, the aim of this study was to investigate
91 the potential of using Hibiscus plant as a primary coagulant and as a coagulant aid, and to
92 assess the floc characteristics in terms of floc size, strength, and recovery ability.

93

94 **2.0 Materials and methods**

95 2.1 Collection and preparation of the seeds

96 All the seeds used in this study, OK, KE and SB, were obtained from a local market in
97 Nigeria. The seeds were manually prepared by removing the seeds from the capsules and
98 pods to access the seed kernels. The seeds were cleaned by washing with tap water to remove
99 contaminants such as stones, plant debris and dust and then dried in an oven at 60°C for six
100 hours. The dried seeds were ground into a fine powder for 2 minutes using a Tema laboratory
101 disc mill. The ground seed powders were then sieved and the powder retained in the 212 µm,
102 and 300 µm sieve sizes was combined and subsequently used in the preparation of the
103 coagulants.

104

105 2.2 Chemicals and reagents

106 Analytical grade sodium chloride, aluminium sulphate and hydrochloric acid (Fisher
107 Scientific, UK), kaolin Fluka-60609, (Sigma-Aldrich, Germany), sodium phosphate
108 monobasic monohydrate (Sigma-Aldrich, Germany), and sodium phosphate dibasic (Sigma-
109 Aldrich, UK) were used in the study. Deionized (DI) water was used to prepare all
110 suspensions and concentration solutions.

111

112 2.3 Preparation and extraction of the natural seed coagulants

113 1M sodium chloride (NaCl) solution was prepared by dissolving 58.5 g NaCl in 1000 ml of
114 DI water to obtain the required concentration. The crude seed extract (CSEs) were prepared
115 from the ground seed powders by adding 1.0 M NaCl solutions to the seed powder to make
116 2% (w/v) suspension. The suspension was stirred vigorously using a magnetic stirrer for
117 15min at room temperature (19±2°C). The suspension was then centrifuged at 4500 rpm for
118 10 minutes using a Heraeus Megafuge16 (Thermo Scientific, Germany). The suspension was
119 decanted and the residual solids dried in an oven at 50°C overnight. The weight of the dried
120 solid material was measured to ascertain the amount of seed powder used in making the

121 suspension. The decanted suspension was then filtered through a Whatman No. 42 filter
122 paper. The filtrates were termed crude extracts and were then used as primary coagulant or
123 coagulant aids in a series of jar test experiments.

124 2 g of AS powder was dissolved in 100 ml of DI water and the suspension rapidly mixed for
125 15 minutes, using a magnetic stirrer. This AS coagulant was applied in the jar test
126 experiments to determine the optimum coagulant dose required in the strength test.

127 2.4 Protein purification and lipid extraction from the seed

128 The ground seed powders (212 μ m–300 μ m) were defatted using high-grade hexane in an
129 electro-thermal Soxhlet extractor. 20g of the seed powder was used during the extraction. For
130 efficient extraction, 2L of solvent volume (hexane) was used and heated to 60 °C. The
131 process was run continually for 8 hours with each complete cycle taking 2 to 3 minutes. The
132 residues were dried overnight at room temperature (19 \pm 2°C) and the dried residue was
133 ground into a fine powder using pestle and mortar and was applied in the subsequent
134 purification processes.

135

136 2.4.1 Purification by ion exchange column chromatography

137 A HiTrap Q HP (1 ml) anion column, (GE Healthcare, Sweden) was used for the purification
138 of the protein of interest of the hibiscus plants. The column connected to a pump (Watson-
139 Marlow Breeder pump 323, UK), and the pump head adjusted to a flow rate of 1 ml per
140 minute. The preservatives were washed with 10ml of DI water, followed by ten column
141 volumes (CV) of 1 M NaCl dissolved in the phosphate buffer. The column was then
142 equilibrated with the phosphate buffer 10 CV before loading the protein. 5g of the oil-free
143 powder was dissolved in 0.1 M phosphate buffer and mixed thoroughly for one hour using a

144 magnetic stirrer. The mixture was centrifuged at 20,000 rpm at 4°C for 40 minutes before
145 decanting the supernatant. The supernatant was injected using a peristaltic pump onto the ion
146 exchange column to separate the protein of interest from the contaminants.

147 The sample was loaded at a flow rate of 1 ml per minute, where the protein of interest was
148 bound to the Column matrix throughout the loading process. The weakly bound contaminants
149 were washed away with the equilibrating (initial) buffer using 10 CV. The proteins of interest
150 were eluted, beginning with, 0.3, 0.5 and 1.0 M of NaCl-phosphate buffers and the various
151 fractions collected. The collected fractions were analysed for absorbance using a
152 spectrophotometer (Varian Carey 50 probe UV-visible, Australia) and coagulation
153 performance using a standard jar tester (Phipps and Bird, 7790-900B USA). The purified
154 protein contents were evaluated for floc strength using a laser diffraction particle size
155 analyser (Mastersizer, Malvern 2000, UK).

156

157 2.5 Preparation of the synthetic turbid water

158 Turbid water samples for the jar test experiments were prepared by adding kaolin particles to
159 tap water. 40 g of laboratory grade kaolin (Fluka and high-grade, Sigma-Aldrich) was added
160 to 400ml of tap water, and the suspension stirred for 30min using a magnetic stirrer. The
161 suspension was made up to 1L by adding 600ml of tap water and then stirred for a further
162 30min. The suspension was allowed to stand for 24hr for the kaolin to hydrate and then
163 allowed to stand for another seven days. The supernatant was decanted, and 0.3ml of the
164 stock solution was mixed with 1L of tap water to produce turbidity value ranges of $46 \pm$
165 1NTU.

166 2.6 Jar test experiments

167 Jar tests were conducted using a standard apparatus (Phipps and Bird, 7790-900B, USA)
168 comprising six 1L beakers to evaluate the optimum coagulant dose for the coagulation tests.

169 For effective dispersion of the coagulant, the water was rapidly mixed at 200 rpm for 1.5
170 minutes during which time various doses of the coagulant were added to the beakers. The
171 mixing speed was then reduced to 30rpm for a further 25 minutes to simulate the flocculation
172 stage. The suspension was then allowed to stand undisturbed for 1 hour to facilitate
173 settlement. The long sedimentation time was adopted in order to assess the effectiveness of
174 the process and to see whether the requirement to filter might be avoided after prolonged
175 settlement for people in rural areas. A final treated water sample (10 ml) was drawn via
176 syringe 2cm from the top surface of the water in the beakers. Both initial and final water
177 turbidity were then measured using a turbidity meter (HI 93703, Hanna). In a separate
178 experiment using river water, residual dissolved organic carbon (DOC) was measured in
179 water before and after treatment with crude and purified samples. DOC measurement was
180 conducted using TOC analyser (Shimadzu TOC-V-CSH). All experiments were performed at
181 room temperature ($19 \pm 2^\circ\text{C}$).

182 2.7 Floc formation, breakage and reformation experiments

183 To assess floc regrowth, flocs were broken by introducing rapid mixing at 200 rpm for 1.5
184 min. The rotor speed was then reduced to 30 rpm for 25 min to determine the floc re-growth
185 capability of the various coagulants. To compare the flocculation capacity of the seed extracts
186 as coagulant aids, a predetermined dose was added before the end of the coagulation test with
187 AS as a primary coagulant (i.e. 45s after the AS was dosed).

188 Floc growth, breakage and re-growth were assessed using a laser diffraction instrument
189 (Mastersizer 2000, Malvern, UK), following (Jarvis et al., 2005b, Li et al., 2007, Yu et al.,
190 2012). The Mastersizer was connected to a jar test apparatus and the liquid suspension was
191 monitored by continuously drawing water through the optical unit of the Mastersizer and
192 returning to the jar tester, as shown in Fig. 1.



193

194 **Fig 1 Systematic connection of the Mastersizer 2000 and a jar test apparatus for floc properties**
195 **monitoring.**

196 Pumping was via a peristaltic pump (Watson-Marlow, 323S, USA) positioned on the return
197 tube with 4.8 mm internal diameter peristaltic pump tubing. The inflow and the outflow were
198 located 10 mm above the blade of the jar tester and opposite each other. Measurements were
199 taken every 35s for the duration of the experiment, and the results automatically logged onto
200 a computer. The flow rate was kept at 2 L/hr (i.e. @33.3 ml/min) throughout the experiment
201 to avoid either floc breakage or floc settling in the tubing. The coagulant dosage was the dose
202 obtained from the earlier jar test results as described above.

203 2.7.1 Floc strength and floc recovery factors

204 To understand the properties of the coagulated flocs, it is important to consider the floc
205 strength and floc recovery after exposure to high shear. Floc strength reveals the resistance of
206 the flocs to stress and can be described using a strength factor. Similarly, the recovery factor
207 reveals the ability of a floc to re-grow after breakage. Floc strength and recovery factors were
208 calculated using Equations 1 and 2 following (Sun et al., 2011, Xiao et al., 2011, Yu et al.,
209 2014):

210 $S_f = \frac{d_2}{d_1} \times 100$ Eq. 1

211 $R_f = \frac{d_3 - d_2}{d_1 - d_2} \times 100$ Eq. 2

212 where d_1 is the average median floc size established at the steady phase before breakage, d_2 is
213 the median floc size achieved after it was subjected to high shear rate. The average median
214 size, d_3 , is the average median floc size achieved at the final steady phase after floc breakage.

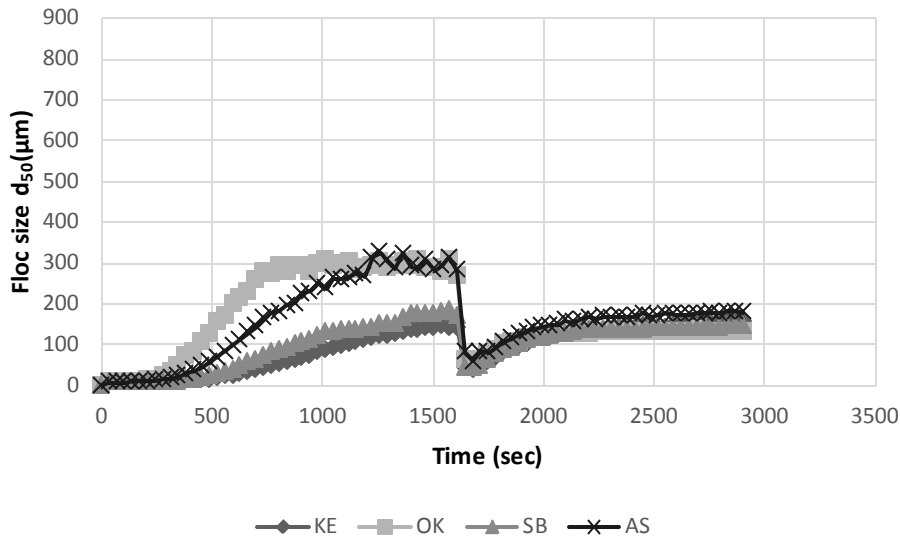
215 **3.0 Results and discussion**

216 3.1 Floc growth and size of Hibiscus seed extracts as primary coagulants

217 The results of the floc formation and breakage experiments using crude extracts of OK, SB
218 and KE and AS as primary coagulants are shown in Fig 2. 50 mg/l dose of each extract was
219 employed as primary coagulants in this work, and the median d_{50} floc size was considered
220 throughout the study. The concentration of proteins in the extracts was 1.018 mg/ml in OK,
221 0.918 mg/ml in SB and 0.631 in KE respectively. Fig 2 shows that the median floc sizes for
222 SB and KE were approximately 176 μ m and 142 μ m respectively, lower than the 300 μ m floc
223 size generated by AS as primary coagulants before breakage. While OK and AS achieved
224 their largest floc sizes after reaching the steady growth phase, it is likely that SB and KE flocs
225 were yet to reach the steady state when the high shear was reintroduced. Furthermore, the
226 growth rate in SB and KE was found to be slower (Fig 2); hence, their flocs needed more
227 time to reach the steady phase before a clearer comparison can be made between the floc
228 sizes. It is clear that the performance of SB and KE extracts as primary coagulants in terms of
229 floc growth was very poor compared with AS flocs due to slow growth rate. Generally, floc
230 growth rates achieved by the crude extracts as primary coagulants were very slow as seen in
231 Fig. 2. This is because the NOM contents, especially the lipid content in the seeds has the
232 potential to coat the surfaces of the flocs (Harold, 2001, Eman N et al., 2010). Previously,

233 (Xiao et al., 2011) have shown that NOM can impede the aggregation of flocs in kaolin-
234 humic substance water sample. However, the floc growth rate was much faster in OK than in
235 AS, demonstrating a shorter period to achieve maximum floc size, probably due to the high
236 inter-particle bridging capability of the extract, which in real terms could result in lower cost
237 of water treatment (Zhao et al., 2013b). In addition, when used as a primary coagulant, the
238 OK sample exhibited good performance achieving the same floc size as that obtained by AS,
239 (approximately 300 μ m). However, under these flocculation conditions, OK produced larger
240 floc sizes due to its high protein concentration, 25% as reported by (Oyelade et al., 2003),
241 compared to SB and KE seed with a lower protein content of 18.8% (Rao, 1996) and 13.04%
242 (Mariod et al., 2010) respectively. Additionally, the high bridging action in OK may have
243 been caused by the 5.09 mg of its protein in the 50 mg/l extract that was used for coagulation
244 while in SB and KE, the amount of protein used for coagulation was 4.59 mg and 3.16mg
245 respectively in the 50 mg/l extract.

246 Several studies have indicated that the main agent of coagulation in natural extracts is the
247 presence of dimeric cationic protein with molecular mass of 6.5 and 14kDa (Ndabigengesere
248 et al., 1995, Ghebremichael et al., 2005, Bodlund et al., 2014).



249

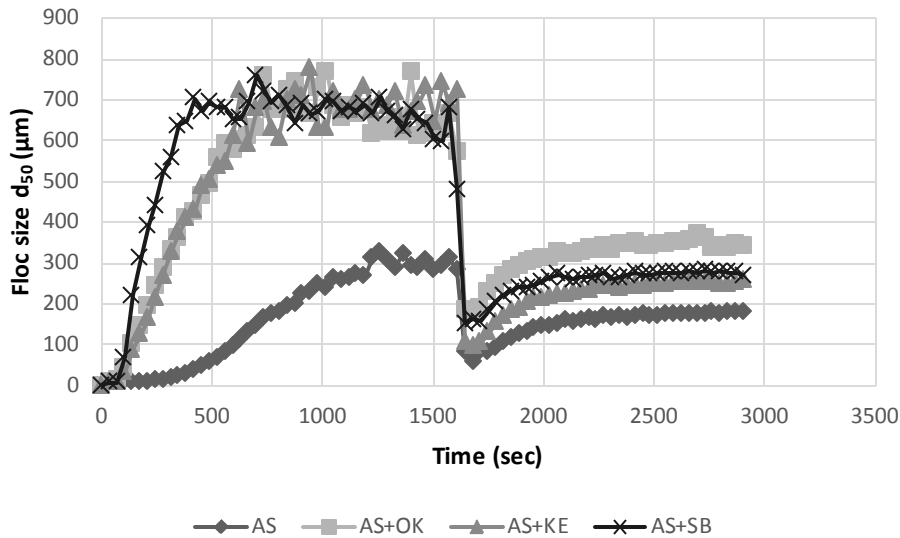
250 Fig 2 Floc growth, breakage and re-growth of KE, OK, SB extracts and AS used as primary coagulants

251 3.2 Floc growth and size of Hibiscus seed extracts as coagulant aids

252 The growth, breakage and re-growth factor of AS and AS+extracts flocs were evaluated as
 253 shown in Fig 3. The AS dose was 5 mg/l, as determined from preliminary jar test experiment.
 254 Similarly, 50 mg/l of each extract was employed as coagulant aids in this work. The results
 255 show that floc growth was influenced greatly by the use of crude extract samples, the effect
 256 being to increase the effective particle collision radius to give greater contact opportunity.
 257 The floc growth patterns were found to be similar for all the extracts for the duration of the
 258 experiments. It appears that floc growth of AS+OK, AS+KE and AS+SB assume a rapid
 259 growth within a few minutes of the coagulation process, although the growth was faster in
 260 AS+SB extract than in AS+KE and AS+OK samples. At steady state, when used as coagulant
 261 aids, SB, KE and OK produced floc sizes of 696µm, 701µm and 722µm respectively, but did
 262 not re-grow to their original sizes after breakage. It is believed here that the organic matter
 263 contents in the seed extracts which affected floc growth in Fig 2, was removed by employing
 264 AS as a primary coagulant. Matilainen et al. (2005) showed that AS is capable of removing
 265 up to 95% of high molecular weight NOM in water. Comparing the floc size of AS+extracts

266 and AS alone in Fig. 3, the d_{50} values for the AS+extract combinations were more than twice
267 the floc size of AS used as primary coagulant (approximately 300 μ m). The increase in size of
268 the AS+extract combination compared to AS alone is attributed to the double action of
269 charge neutralisation and adsorption of AS, being further enhanced by the bridging effect of
270 the extracts. The extracts consist of several protein molecules that contain coagulation
271 compounds that are not limited to charge neutralisation only but exhibit adsorption and
272 bridging also (Zhao et al., 2013a), which give rise to increased floc growth. It is believed that
273 the most important coagulation mechanism here involves charge neutralisation and patchwise
274 adsorption of AS, which later provides adsorption sites for the extracts to form bridges with
275 the other particles. It is clear that the addition of AS first then followed by the extracts
276 provided the most effective flocculation process to increase floc size. This result is in
277 agreement with work reported by Yu et al. (2009), who observed that flocs formed by charge
278 neutralisation and bridging action are larger than flocs generated by simple charge
279 neutralisation. The AS floc size reported in this study (approximately 300 μ m) is the same as
280 that obtained by (Zhao et al., 2013b), who used *Enteromorpha* extract as a coagulant.

281



282

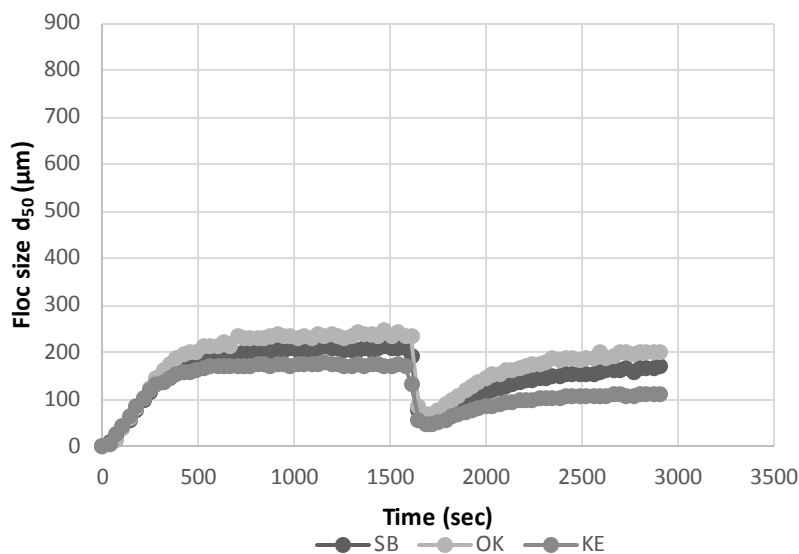
283 **Fig. 3 Floc growth, breakage and re-growth of OK, KE and SB extracts used as coagulant aids**

284

285 However, particle concentration in water has an impact on floc growth and size since the rate
 286 of adsorption and bridging by natural extract increases as particle concentration increases.
 287 For instance, Muyibi and Evison (1995) and Ndabigengesere et al. (1995) reported in
 288 separate studies that MO extract was found to be ineffective in coagulating low turbidity
 289 water. Similarly, Lee et al. (2001) used a low molecular weight polymer (10 and 50 kDa) as
 290 primary coagulant and observed that the polymer was more effective in the treatment of water
 291 with high turbidity. Thus the size of the floc in the work reported here may have increased
 292 beyond that size if higher turbidity water had been used. However, water samples with higher
 293 turbidity than the one used here were found to affect the measurement due to light
 294 obscuration. Furthermore, the presence of many macro-molecules in the extracts with
 295 different MW proteins and polysaccharides with long carbon chains may be responsible for
 296 particle bridging. Such polymeric chains can effectively absorb colloids through absorption
 297 and bridging effects as in Fig. 3, which resulted in the formation of larger flocs.

298 To examine the impact of low pH coagulation, the growth, breakage and re-growth of flocs
 299 formed by OK, SB and KE seed extracts at pH 4 are presented in Fig 4. At lower pH, the

300 average floc sizes of SB and KE were observed to be approximately 210 μm and 174 μm
 301 respectively; i.e. larger than their corresponding floc sizes of 176 μm and 142 μm when used
 302 as primary coagulants at neutral pH. However, during the same growth period, the d_{50} floc
 303 size of OK decreased from 300 μm to 240 μm at pH 4. One primary cause of the decrease in
 304 floc size in OK is thought to be due to the high lipid content in the seed and partly that there
 305 may be proteins in OK seed that are sensitive to pH change and so deteriorated at low pH.
 306 The change in pH may have caused a change in the protonation pattern of the proteins
 307 especially at lower pH where protein configuration changes. Conversely, the increase in
 308 average d_{50} floc size in SB and KE is attributed to improved coagulation efficiency between
 309 the colloids and the coagulants at low pH, because kaolin particles have been reported to be
 310 less negatively charged at pH lower than neutral (Yin, 2010).



311

312 Fig 4 Floc growth, breakage and re-growth using OK, SB and KE crude extracts at pH 4.

313 3.3 The size of re-grown flocs of Hibiscus seed extracts

314 The d_{50} values for all samples were found to decrease rapidly with the re-introduction of the
 315 high shear rate at 200 rpm. The flocs began to re-grow when the slow mixing was re-
 316 introduced. Examination of Fig. 2 shows that when the extracts were used as primary

317 coagulants, whilst the size of the re-grown flocs were almost the same, (approximately
318 146 μ m), the breakage was most severe in OK due to its high organic matter content, because
319 flocs formed under such conditions are more fragile (Jarvis et al., 2005b). Furthermore, flocs
320 generated by KE possess a higher strength factor than flocs produced by OK or SB under the
321 same experimental condition. It is possible in this case that the ligand-binding site in KE has
322 higher affinity than those of OK and SB seeds, which provide stronger bonding with other
323 molecules. As re-growth floc sizes were similar across all the extracts, it is postulated that the
324 breakage force may have induced similar changes on the floc properties because of
325 similarities in amino acid sequence in the seeds and therefore the charge re-distribution
326 resulted in similar floc re-growth. Therefore, the use of the extract as primary coagulants is
327 not technically beneficial except that, it is affordable and easy to process to low income
328 countries.

329 Fig. 3 shows that as coagulant aids, AS+OK produced the largest regrown floc size of 350 μ m
330 at steady state, compared to 280 μ m and 274 μ m for AS+SB and AS+KE respectively. The
331 difference in size of the regrown flocs may have been caused by charge re-distribution after
332 breakage. As a result, each extract took a different pattern of floc re-growth which could be
333 linked to individual peptide structure (bonding sequence) of the extract. The results show no
334 significant difference between the AS+SB and AS+KE flocs at steady state before and after
335 breakage. The behaviour and response of the samples to the breaking force was similar but
336 more extensive in AS+KE followed by AS+SB whereas the amount of breakage recorded in
337 AS+OK was found to be lower. The performance of AS+OK was superior to that of AS+SB
338 and AS+KE after floc breakage, due to the high protein concentration in OK. Such behaviour
339 confirms that all the samples may possess similar protein compounds, although they may
340 differ in composition and coagulation activity.

341

342 3.4 Floc strength and recovery factors of Hibiscus seed crude extracts

343 Table 1 summarises floc strength and recovery ability, using coagulants extracted from
344 Hibiscus seeds. The results show that as coagulant aids, OK exhibited the highest strength
345 (25.5%) exceeding KE and SB (21.8% and 15.0% respectively). While floc strength factor
346 increased from 21.8% to 33.8% in KE and 15% to 25.0% in SB, the strength of the OK-
347 derived flocs deteriorated from when used as coagulant aid to when used as primary
348 coagulant. The low lipid contents in SB and KE extracts are thought to have helped in
349 improving the inter-particle bonding resulting in higher strength factors. Conversely, the
350 decrease in floc strength from (25.0% to 23.3%) in OK, is thought to be due to the presence
351 of high lipid content in the seed which can inhibit inter-particle bonding due to lack of
352 bridging action (Eman N et al., 2010, Sharp et al., 2006a). After floc breakage, a notable floc
353 recovery was seen in both SB and KE as primary coagulants (100% and 76.5%, respectively).
354 Following its low floc strength performance, OK again showed a corresponding poor floc
355 regrowth by recovering only 32.6% of its original floc size. Further evaluation of the flocs
356 after breakage when OK, SB and KE were used as coagulant aids shows that, OK recorded
357 the highest floc recovery factor of 38.6% compared with 26.6% and 23.5% recovery ability
358 recorded by SB and KE respectively. The high recovery ability in OK which coincides with
359 its high strength factor when used as coagulant aid is likely to be due to the combine effect of
360 AS plus the high protein content in the seed which improve the charge neutralisation and
361 bridging action. The work reported here observed a direct relationship between strength
362 factor and recovery factor in both OK and KE extracts. The results show that a poor floc
363 strength factor led to limited floc recovery, whereas stronger flocs exhibited a level of
364 significant floc re-growth.

365 Table 1 shows that there was very little difference between the floc strength in OK and KE
366 extracts at low pH (29.3% in OK, 28.7% in KE and 33.3% in SB). It is clear that the acidic

367 pH value played an important role in improving the strength of OK and SB, enabling
 368 adsorption sites for tighter bonding but slower floc growth, especially in OK. However, the
 369 floc strength of KE at pH 4 when used as a primary coagulant is reduced from (33.8% to
 370 28.7%). Table 1 further shows the re-growth of flocs formed by the three samples at low pH
 371 after floc breakage, indicating a recovery ability of 46.8% for KE, 65.7% for SB and 75.7%
 372 for OK. In separate studies, Cao et al. (2010) and Sun et al. (2011) reported that flocs formed
 373 in acidic pH region were stronger and more recoverable than flocs generated in alkaline
 374 conditions. However, despite the high floc strength of 33.3% recorded at pH4 by SB, floc re-
 375 growth was 65.7%; i.e. lower than the 100% floc recovery ability recorded when its floc
 376 strength was 25% as primary coagulant. The cause of this is likely to be due to a change in
 377 protonation pattern of SB protein at low pH which affected its binding activity during floc re-
 378 growth.

379

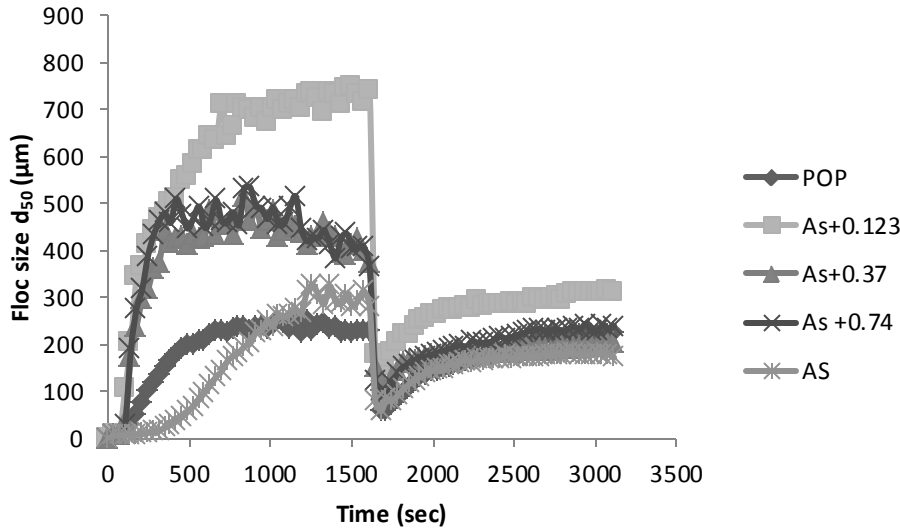
380 Table 1 Characteristics of floc strength and recovery factor of crude extract used as primary coagulants and as coagulant aids

Parameters	Crude coagulant		
	OK	SB	KE
Strength factor (%)			
• CE+AS @ neutral pH	25.5	15.0	21.8
• CE @ neutral pH	23.3	25.0	33.8
• CE @ pH4	29.3	33.3	28.7
Recovery factor (%)			
• CE+AS @ neutral pH	38.6	26.6	23.5
• CE @ neutral pH	32.6	100	76.5
• CE @ pH4	75.7	65.7	46.8

381

382 3.5 Floc growth and size of purified Hibiscus seed as coagulants and as coagulant aids

383 The performance of purified protein samples on floc growth and size as primary coagulants
384 or as coagulant aids are presented in Figs 5, 6 and 7. In the work reported here, coagulant
385 protein doses used in the experiments were 0.123, 0.37 and 0.74 mg/L with a pre-determined
386 AS dosage of 5mg/L obtained from preliminary jar test results. The concentration of each of
387 the purified proteins used in the study was found to 1.238 in POP, 1.211 in PSP and 1.092
388 mg/ml in PKP respectively. Fig 5 shows that the largest floc size of approximately 741 μ m
389 was recorded when 0.123 mg/l of POP was added to 5 mg/l of AS, as coagulant aid. It is
390 clear, therefore, that the purification of OK seed proteins greatly improves its performance as
391 a coagulant aid. Further increase in POP dose from 0.123 mg/l to (0.37 and 0.74mg/L) led to
392 a decrease in floc size, producing median d_{50} floc sizes of 490 μ m and 502 μ m respectively.
393 This decrease in floc size is attributed to the release of excessive charged species from the
394 combined effects of AS and POP needed for effective charge neutralisation, adsorption and
395 bridging flocculation to occur. It is essential for successful bridging to occur using natural
396 coagulant that sufficient particles with available unoccupied surfaces are present in order to
397 facilitate polymer chains attachment that are adsorbed on other particles (Bolto and Gregory,
398 2007). In this case, subsequent addition of the coagulant proteins+AS reduced the available
399 particle surfaces for charge neutralisation, resulting in insufficient adsorption sites for inter-
400 particle bridging. These conditions of increasing coagulant aid dose to 0.37 and 0.74 mg/l
401 resulted in the formation of smaller floc sizes. Interestingly, the re-growth ability of flocs
402 formed from water coagulated with 0.37 and 0.74 mg/l of POP as coagulant aid, and when
403 POP was used as primary coagulant, was much lower than when 0.123 mg/l was employed as
404 a coagulant aid. Overall, the floc sizes attained were approximately 300 μ m with 0.123 mg/l,
405 196 μ m with 0.37 mg/l, and 232 μ m in both 0.74 mg/l and POP despite their small pre-
406 breakage floc sizes.

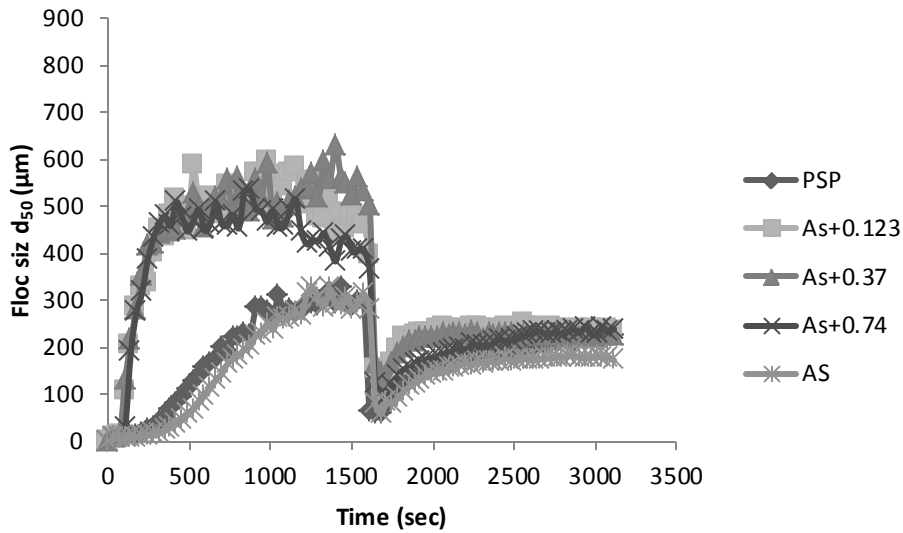


407

408 **Fig 5 Floc growth, breakage and re-growth of POP used as primary coagulant and as coagulant aids**

409 Fig 6 shows the growth, breakage and re-growth of aggregated floc formed by PSP. A faster
 410 initial floc growth rate was exhibited by the PSP sample with coagulant aid doses of 0.123,
 411 0.37 and 0.74 mg/l in combination with AS than when used as a primary coagulant. At steady
 412 state, maximum floc sizes, d_{50} of $580\mu\text{m}$ and $519\mu\text{m}$ were achieved with 0.123 mg/l and 0.37
 413 mg/l doses respectively. The flocs generated with 0.74 mg/l of PSP in conjunction with AS
 414 were weaker and smaller in size, producing $491\mu\text{m}$ diameter flocs. This reduced floc size is
 415 largely due to saturation of polymer bridging sites caused by the additional coagulant dose.
 416 At steady state, PSP assumed a different pattern of floc growth, where the absolute deviation
 417 of the median floc size about the mean value was found to be greater than in flocs generated
 418 by POP. However, the pattern taken by the regrown flocs was similar for all samples
 419 regardless of coagulant aid dosage and also irrespective of pre-breakage floc size. During
 420 flocculation, thread-like flocs, visible to the naked eye under lamination, grow in length and
 421 circumference. At the end of the measurement period, the regrown flocs reached a steady
 422 phase d_{50} floc size of $243\mu\text{m}$ in all the samples, including flocs formed by PSP as primary
 423 coagulant. It is noteworthy that, when used as primary coagulant, PSP produced an initial
 424 median floc size, ranging between 295 and $300\mu\text{m}$ similar to the floc generated by AS as

425 coagulant, (approximately 300 μm). However, the ionic strength of SB species was not
426 sufficient to compress the double layer during coagulation as revealed by its surface charge
427 potential.

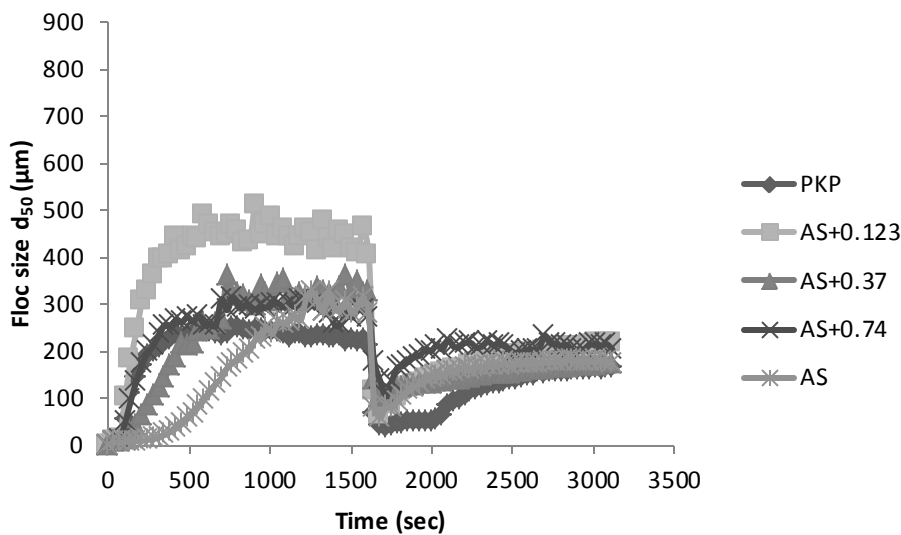


428

429 **Fig 6 Floc growth, breakage and re-growth of PSP used as primary coagulant and as coagulant aids**

430 Fig 7 shows floc growth, breakage and re-growth performance when using PKP as coagulant
431 and as a coagulant aid. A maximum median floc size of 480 μm was recorded when 0.123
432 mg/l of PKP was used as coagulant aid in conjunction with AS. The results show a decrease
433 in floc size as the dosage of the coagulant aid increased, similar to the trend of floc growth
434 shown by POP and PSP. A maximum floc size of 335 μm was recorded with 0.37 mg/l of
435 PKP and 310 μm diameter floc size was generated with 0.74 mg/l dose. This again is due to
436 insufficient adsorption sites as most of the available particle surfaces are covered with
437 increased coagulant addition. This situation can be overcome by improving bridging
438 flocculation conditions. La Mer (1966) postulated that optimum dosage corresponds to half of
439 the particle surface coverage. Hence, understanding the surface charge potential in a system
440 plays an important role in achieving enhanced floc formation during flocculation. Again, the
441 recovery ability of floc generated by 0.37 and 0.74 mg/l of PKP as coagulant aid and PKP as

442 primary coagulant was found to be higher than floc recovered by 0.123 mg/l of PKP used as
 443 coagulant aid. While the d_{50} size of the regrown floc was 201 μm with 0.123 mg/l dose of
 444 PKP, the 0.37 and 0.74 mg/l doses achieved post-breakage steady d_{50} sizes of 164 μm and
 445 211 μm respectively. Thus, as coagulant aid, PKP exhibited the greatest floc strength and re-
 446 growth capability at a higher dose, although the initial floc size was smaller. There was a
 447 modest, yet noticeable, amount of thread-like flocs using PKP, but this was less than that
 448 observed in PSP flocs, indicating that the two seeds may have linked amino acid
 449 characteristics.



450

451 **Fig 7 Floc growth, breakage and re-growth of PKP used as primary coagulant and as coagulant aids**

452 **3.6 Floc strength and recovery using purified Hibiscus seed proteins**

453 Table 2 presents floc strength and recovery of the purified hibiscus proteins used as primary
 454 coagulants and as coagulant aids. When the purified seed proteins were used as primary
 455 coagulants, the results show that the highest strength factor of 24.3% was recorded for POP,
 456 21.7% for PSP and 18.2 % for PKP while flocs formed by AS had a strength factor of
 457 approximately 20%. In addition, the results show that flocs formed with POP and PSP dosed
 458 as coagulant aids can resist marginally higher shear with a 0.123 mg/l dose compared to AS

459 at 5mg/l dosage under the same coagulation conditions. The high floc strength recorded by
 460 the two purified samples is thought to be due to the protein contents and their sequence in the
 461 seeds with a higher affinity to bind other molecules. The presence of many macromolecules
 462 from natural extracts is reported elsewhere to be associated with adsorption and bridging
 463 action which is believed to be the main agent for the coagulation activity (Antov et al., 2010).
 464 Furthermore, the addition of the 0.123 mg/l dose as coagulant aid with AS produced larger
 465 floc size with corresponding decrease in floc strength of POP and PKP, whereas the floc
 466 strength of PSP remain largely unchanged . This results agrees with (Jarvis et al., 2005a) who
 467 observed that the resistance of smaller flocs in turbulent flow regions is higher than that of
 468 larger flocs.

469 **Table 2 Characteristics of floc strength and recovery factor of purified proteins used as primary and as**
 470 **coagulant aids**

Parameters	POP	PSP	PKP	AS
Strength factor (%)				
• Primary coagulant	24.3	21.7	18.2	20
• AS + 0.123 mg/l	20.8	21.4	14.0	—
• AS + 0.37 mg/l	20.9	22.2	31.3	—
• AS + 0.74 mg/l	19.2	19.5	35.6	—
Recovery factor (%)				
• Primary coagulant	70.7	71.4	64.3	50
• AS + 0.123 mg/l	27.3	25.7	38.0	—
• AS + 0.37 mg/l	28.4	25.2	25.1	—
• As + 0.74 mg/l	36.9	31.4	59.0	—

471 Interestingly, when the coagulant dose was increased from 0.123 to 0.37 mg/l, and
 472 maintaining the same shear rate, the strength factor was broadly the same for POP (20.8% to
 473 20.9%) but increased slightly for PSP from 21.4% to 22.2%. In the case of PKP, the increase
 474 was significantly higher, from 14.0% to 31.3%. Further increase in coagulant aid dose to 0.74
 475 mg/l caused further floc strength decline in POP and PSP. It is thought that a lack of proper

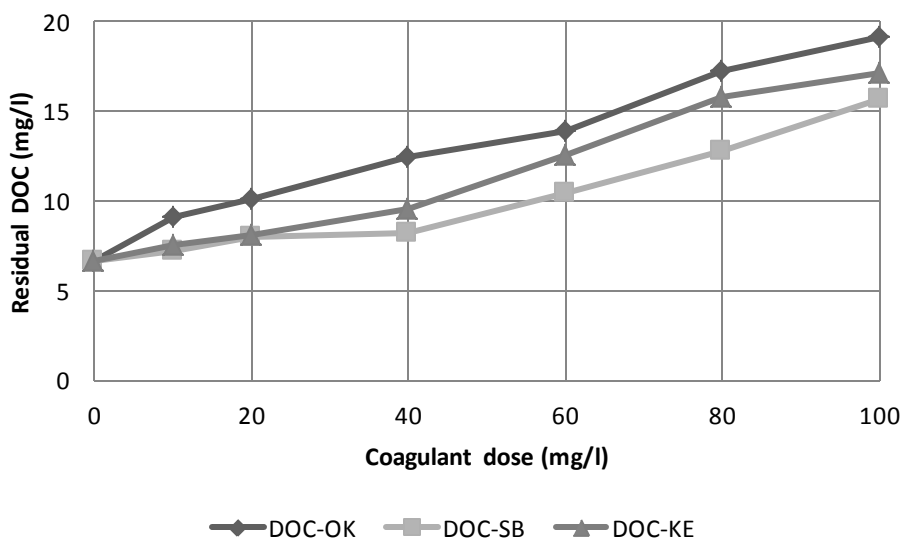
476 initial bonding due to polymer saturation may have been the major cause of this trend in floc
477 strength deterioration. Under the same conditions, floc strength improved further in PKP
478 from (31.3% to 35.6%). PKP flocs were found to behave in a similar fashion to AS in work
479 reported by (Yu et al., 2014), who showed that an increase in alum dose during coagulation
480 resulted in increased floc strength. The work reported here noted that an increase in PKP
481 dosage from 0.123 to 0.74 mg/l resulted in a further increase in floc strength factor, from 18.2
482 to 36.0%. Although PKP has low protein content as reported earlier, flocs generated by 0.37
483 and 0.74 mg/l PKP were stronger than flocs formed by POP and PSP under the same dosage
484 condition. The result demonstrated that if PKP is used as coagulant aid with AS, at a higher
485 dose of 0.74mg/l, the improvement in floc strength was significantly higher compared with
486 0.123 and 0.37 mg/l doses. Further investigation revealed that floc reversibility of the purified
487 proteins was better when the samples were used as primary coagulants, with PSP, POP and
488 PKP re-growing to 71.4%, 70.7% and 64.3% of their original size respectively, whereas AS
489 flocs recovered only 50% of their original size. The slight difference in floc recovery ability
490 in PKP may be attributed to its low protein content of 10.56% as reported by (Mariod et al.,
491 2010). However, there is a clear indication that all the seeds have some similarity in their
492 amino acid sequence, since they are of the same species, and the redistribution of the surface
493 charge after breakage took a broadly similar pattern. Furthermore, at a higher coagulant aid
494 dose of 0.74 mg/l, the recovery factor improved across all the samples compared to 0.123
495 mg/l dose. While floc recovery ability was 59.0% in PKP and 36.9% in POP, the recovery
496 factor was only 31.4% in PSP which was lower than the recovery ability recorded by the
497 other samples. Again, at the 0.123 mg/l dose, floc recovery by the PKP sample was much
498 higher than the maximum floc re-growth achieved by PSP and POP at 0.74 mg/l as coagulant
499 aid. It is noteworthy, however, that the re-growth of PSP and PKP flocs was the same,
500 (approximately 25% at 0.37 mg/l dose) while the regrown floc was 28.4% in POP.
501 Nevertheless, all flocs generated by POP and PKP as coagulant aids achieved higher floc

502 recovery factors than PSP flocs under the same shear force condition, probably as a result of
503 the thread-like flocs formed by PSP being easily broken.

504

505 3.7 Effect of DOC in treated water using Hibiscus plants

506 Figure 8 shows the residual DOC concentration when water was dosed with specific Hibiscus
507 crude extract concentrations. The result reported in this work is in agreement with several
508 previous research studies (Ndabigengesere and Subba Narasiah, 1998, Okuda et al., 2001)
509 where DOC addition in final water was found to be significant. The DOC concentration
510 increased from 6.7 mg/l in raw water to 19.1, 15.7 and 17.1 mg/l when dosed with 100 mg/l
511 of OK, SB and KE, respectively. Crude extracts may contain compounds other than proteins
512 such as fats carbohydrate, fibre etc. which impacted the overall water treatment quality. The
513 organic matter from the extracts may be a surrogate for disinfection by-products (DBPs)
514 formation if chlorine is used (Liu et al., 2014).

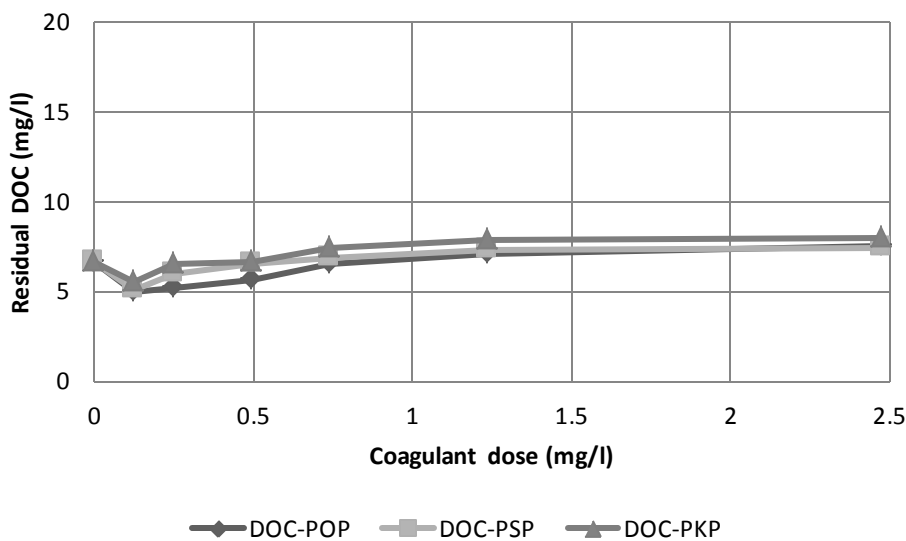


515

516 **Fig 8 Impact of DOC additions in treated water using OK, SB and KE seed extracts.**

517

518 Similarly, Figure 9 shows the performance of the purified proteins in terms of DOC
 519 concentration in the clarified water. It is noteworthy that the use of POP, PSP and PSP
 520 lowered the treated water DOC in the final water. At optimum doses, DOC decreased from
 521 6.7 mg/l to 5.0, 5.1 and 5.5 mg/l in POP, PSP and PKP treated waters. It is clear that the use
 522 of the purified proteins can reduce the impact of DBP formation in water and the purification
 523 process achieved the desired goal of obtaining the proteins in pure state. All the contaminants
 524 in the seeds that may have contributed to increasing the overall organic matter in water were
 525 removed.



526

527 **Fig 9 Impact of DOC in treated water using POP, PSP and PKP as coagulants.**

528

529 **4.0 Conclusions**

- 530 1. When used as a coagulant aid in conjunction with AS as the primary coagulant,
 531 Hibiscus seed extracts can significantly improve floc growth and strength in water
 532 treatment. A doubling of floc size was achieved with a 0.123 mg/l dose of purified
 533 seed proteins. The floc recovery ability of POP, PSP and PKP was found to increase
 534 as coagulant aids doses increased to 0.74 mg/l, but an improved floc re-growth of

535 70.7%, 71.4% and 64.3% was achieved by POP, PSP and PKP when the samples
536 were used as primary coagulants.

537 2. Flocs formed by PKP at a dose of 0.74 mg/l and flocs formed with 0.123 mg/l of PSP
538 were more resistant to breakage than AS flocs, but POP flocs were strongest when it
539 was used as a primary coagulant.

540 3. The application of Hibiscus seeds can help to prevent filter clogging because it
541 generate larger flocs that can settle effectively, especially as coagulant aids. In crude
542 form, SB and KE exhibited excellent re-growth capability (100% and 76.5%,
543 respectively).

544 4. The findings support the hypothesis that the dominant flocculation mechanism for all
545 the extracts was favoured by sorption and bridging action due to the availability of
546 many macro-molecular proteins which are anionic.

547 5. The effects of Hibiscus plant seeds as coagulant aids were clearly demonstrated in this
548 work and so a notable benefit can be derived from its application by people in low
549 income countries, because it is non-toxic and significant cost savings can be achieved
550 in water treatment due to the lower dose of AS requirement.

551

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558 measurements.

559

560 Conflict of interest

561 The authors wish to declare that there are no conflicts of interest regarding the publication of
562 this paper.

563

564 Author's contributions

565 The first author conducted the laboratory works and participated in the analysis of the results
566 and writing up of the paper.

567 The second author participated in reviewing the experimental procedures, data analysis and
568 writing the research paper.

569

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