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Studying effect of water quality parameters on coagulation efficiency by *Moringa Oleifera* seeds

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ABSTRACT

The purpose of this study is to investigate the performance and efficiency of *Moringa* seeds from different sources on turbidity. A protein analysis test was done for each source; then two different methods of extraction were compared to examine the coagulation activity for the Moringa's active ingredient. The results of sodium chloride (NaCl) extraction in comparison to distilled water extraction of the *Moringa oleifera* seeds showed that the salt solution extraction technique was more efficient than the distilled water for extracting the active coagulant ingredient. The above mentioned findings were used to examine the effects of other parameters such as pH, calcium and magnesium hardness, bicarbonate-alkalinity, and salinity independently on turbidity removal with an optimum dosage of 1% NaCl extract of dry shelled *Moringa* seeds. The obtained results showed that the water quality parameters had no significant effect on the coagulation potential of the NaCl extract of the shelled *Moringa* seeds and was almost amenable to a wide range of water environment condition.

1. Introduction

One of the critical factors in water treatment is the reactivity of particles, and accordingly the amenability of these particles to destabilization [1]. In the water treatment industry, turbidity removal can be achieved by a coagulation–flocculation process, which entails the use of coagulant(s), followed by sedimentation and filtration steps. The coagulation process involves the addition of coagulant chemicals to a target water in order to condition it and remove the dissolved matter, colloidal material, and suspended particulates. From the chemical nature point of view, coagulants can be divided into three groups: inorganic (e.g. aluminumsulfate and polyaluminum chloride), synthetic organic polymers (e.g. polyacrylamide derivatives and polyethylene imine), and natural coagulants (e.g. chitosan and microbial coagulants). Aluminum salts are the most commonly used coagulants in water treatment [2,3,4]. Recent research has shown that using aluminum as a coagulant has serious imperfections. Its relationship with Alzheimer's disease [2,3,4], production of high volumes of sludge [5], reaction with alkalinity thus decreasing the pH of the water [6], and low coagulating effect in cold water [7,8] are examples of the problems concomitant to the use of aluminium salts as coagulants. Furthermore, using aluminum in some developing countries corresponds to high treatment costs [9]. In recent years, the developing world has focused on finding and applying appropriate low-cost technologies for treatment of drinking water. A viable solution to such problems would be the local production of any needed treatment materials, either by local production or by exploiting abundant local alternatives [10]. Natural coagulants of mineral and vegetable sources were used by the water and wastewater treatment industry long before the advent of synthetic chemicals like the salts of iron and aluminum [4]. The former have increased potential and as a consequence, many researchers have shown increased interest in them due to their abundance, low prices, innocuousness, multifunction, and biodegradation potential [11].

Moringa oleifera is a tropical plant belonging to the *Moringaceae family* which is a single genus family of shrubs that, so far, has 14 known species of trees [12]. It is known as ben tree, drumstick tree, and by various dialectal names in Africa, India, and Malaya. These species grow rapidly and can survive in bad weather for long periods of time. Moreover, they display varying coagulation potentials [9,13,14]. *Moringa* is renowned as *Gaz Roghani* in southern areas of Iran and even a species of this tree called *Khar Aroos (Mor*

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inga Progeria) is native to Iran [15].

Moringa oleifera has been characterized as a vegetable, medicinal plant, and a source of vegetable oil. Since nearly every part of the tree may be used as food or may have some other beneficial property, *Moringa oleifera* is regarded as one of the world's most useful trees and it is described as a miracle tree [9,16]. In Malaysia, the slender green pods are a staple in their daily diet and the seeds of the brown pods are eaten like peanuts [17].

Numerous studies have reported that using the *Moringa oleifera seeds* have the following advantages: (i) being effective in water softening [18], (ii) having appreciably good, even almost perfect, effects on reducing turbidity of raw water [2,4,9,12,19], (iii) being non-toxic [20], (iv) improving and amending produced sludge [21], and (v) coagulation efficiency of the *Moringa* seeds powder is independent of storage conditions, container types, and duration of storage [3]. The extraction of the active ingredient dimeric protein *from Moringa oleifera* seeds with water has a molecular weight of nearly 13 kDa and an iso-electric point between 10 and 11. Generally, the use of *Moringa oleifera* seeds as the main coagulant, following either distilled water or NaCl solution extraction, to eliminate turbidity from raw waters and synthetic turbid waters indicates that these seeds offer turbidity removal efficiencies within 80% to 98% [2,3,4,9,18].

Distilled water extraction of *Moringa oleifera* seeds appreciably affects the extraction of the active ingredient and, as a consequence, turbidity removal efficiency. Yet, its performance under this scenario lags behind that affected by the salt extraction technique. Okuda et al. [19] reported that the salt solution extract of the *Moringa oleifera* seeds has better coagulation activity and hence, a higher efficiency in the removal of kaolin turbidity in comparison with the distilled water extract. The former has optimum dosages on the average 7.4 times lower than those of the latter. Many researchers have studied the different aspects related to *Moringa oleifera* seed extraction using distilled water in general, and the effects of the water extract on turbidity removal in particular. However, published literature lacks adequate investigation of different conditions and aspects pertaining to the potential for turbidity removal and efficiency using salt solution extracts of the *Moringa oleifera* seeds, especially the effect of water quality parameters on turbidity removal using the *Moringa oleifera* seed extract with 1 mol L^{-1} NaCl. It is proposed that our work may fill this void and contribute to future research works. The objective of the present study is to examine different sources of seeds and compare different extraction techniques. After finding the best source and extraction method, this study will investigate the effects of other water parameters such as pH, alkalinity, hardness, and salinity on turbidity removal.

2. Materials and methods

2.1. Preparation of shelled Moringa oleifera seeds powder

The *Moringa oleifera* seeds used in this study were native to Seri Serdang and Serdang Raya, Malaysia. High quality seeds were taken from the pods and dried in the oven for 24 hours at 50 ºC. The wings and shells of the dry seeds were removed and the kernels were ground and smashed into a fine powder with a mortar and pestle.

2.2. Preparation of shelled Moringa oleifera seeds stock solution

Stock solutions of *Moringa* seed powders were prepared by dissolving 1000 mg of the powder in 40 ml of extractant (distilled water and 1 mol L^{-1} NaCl solution separately).The resulting suspension was mixed using a domestic blender for two minutes at high speed in order to extract its active ingredient. The suspensions were then filtered through muslin cloth and the filtrate collected and made up to 100 ml to produce a stock solution of 10,000 mg L^{-1} powder solution. The working solutions of *Moringa* seed extracts were prepared fresh by appropriate dilutions right before each coagulation test.

2.3. Preparation of synthetic turbid water

Laboratory grade kaolin was used for preparing turbid water samples for all experiment runs. Ten grams of kaolin were added to one liter of distilled water. The suspension was stirred gently at 20 rpm for 1 hour in a jar test apparatus in order to achieve a uniform dispersion of the kaolin particles. The suspension was left standing for 24 hours so as to achieve complete hydration of the kaolin. This kaolin suspension served as the stock solution and was diluted using the distilled water to prepare water samples of 200 NTU.

2.4. Preparation of turbid suspensions of specified/ predefined composition

Five water quality parameters were investigated in this study in terms of their effects on the coagulation function of the *Moringa* seeds. These parameters are pH, calcium and magnesium hardness, salinity, HCO_3^-, and alkalinity. The effects of these parameters on the rate of turbidity removal under an optimum dosage of the *Moringa* were tested at a fixed initial condition and turbidity of 200 NTU. Each of these water quality parameters was applied separately and in seven different doses (25, 50, 75, 100, 150, 200 and 250 mg L^{-1}) of 1 M of each stock solution. In addition, ranges for the studied parameters were widened as much as possible in order to generalize this for the raw water. Also the effects of different pH values (5, 5.5, 6, 6.5, 7, 7.5 and 8) on turbidity removal by the *Moringa oleifera* seeds were investigated under the optimum dosage. This particular pH range was adopted as it complies with the pH values common to most water sources. The pH was adjusted using 0.1 M solutions of laboratory grade sodium hydroxide (R&M, UK) and hydrochloric acid (R&M, UK). The complete list of chemicals which were used in this study to prepare and simulate the water quality parameters is shown in Table 1.

Table 1. List of chemicals used in this study for preparation and simulation of water quality parameters.

Name	Chemical formula	Sources
Sodium hydroxide	$NaOH$	R&M.UK
Hydrochloric acid	HCl	R&M.UK
Sodium bicarbonate	$NaHCO_3$	R&M.UK
Magnesium chloride	$MgCl_2.6H_2O$	R&M.UK
Calcium chloride	$CaCl_2.6H_2O$	R&M.UK
Sodium chloride	$NaCl$	R&M.UK

2.5. Coagulation tests

The coagulation tests were conducted following the jar test which is the method most commonly used for simulating the coagulation-flocculation process in a water treatment plant [4]. A six-place jar test device was employed in the coagulation and flocculation-sedimentation experiments. Six 500-ml beakers were filled with 500 mL each of synthetic turbid water. The operating variables in this study for the jar test were stirring for 4 minutes at 100 rpm (rapid mixing) and for 25 minutes at 40 rpm (slow mixing) followed by 30 min of sedimentation [22,3]. At the end of the sedimentation period, a 30-mL aliquot of the sample was collected from the middle of each beaker for measurement of the residual turbidity using a turbid meter (HACH 2100N, USA).

2.6. Protein evaluation

The protein was estimated according to Bradford's method [23] using a bovine albumin serum (BSA) as a standard solution by an absorbance at 595 nm.

3. Results and discussion

3.1. Effects of different sources of seeds and extraction methods

The effects of different sources of *Moringa* seeds under different extraction methods on turbidity removal were investigated. The two sources investigated in this study were native to Seri-Serdang and Serdang-Raya, Malaysia. Figs. 1 and 2 show optimum dosages of the distilled water and 1 mol L^{-1} NaCl extracts from Seri-Serdang and Serdang-Raya *Moringa oleifera* seeds, respectively. Fig. 1 reveals that when the different sources of seeds were tested under distilled water extract for the same initial turbidity removal efficiency (about 96% for both of them), the optimum dosage of *Moringa* seeds native to Seri-Serdang (140 mg L^{-1}) was two times better than that of the seeds from Serdang-Raya (260 mg L^{-1}). The optimum dosage of the Seri-Serdang seed was 140 mg L^{-1} and 260 mg L^{-1} for the Serdang-Raya seed. It should be mentioned that the turbidity removal in both sources of the seeds was almost the same, 96% for the Seri-Serdang seeds and 95.5% for the Serdang-Raya. Meanwhile, Fig. 2 shows the differences in optimum dosages and consequent residual turbidity between the different seeds extracted using 1 mol L^{-1} NaCl. Since this work investigates distilled water and salt extracts of the seeds which are derived from different sources, the scope of this research is much wider than that of the Ng Shu's [24] who only studied water extracts of *Moringa* seeds from different sources. However, it should be highlighted that despite the differences in optimum doses between the two types of extracts, the ratio of NaCl-extract optimum dose to that of the water extract is the same for both sources of the seeds being equal to two. Moreover, the optimum dosage for NaCl seed extracts of both sources is better than that of the distilled water ones. As can be seen from the optimum dosage results, Serdang Raya seeds extracted by 1 mol L^{-1} NaCl solution have an optimum dosage that is almost three times lower than that of the Serdang Raya seeds extracted by the distilled water and almost 1.75 times lower than that of the Seri-Serdang by the distilled water. Nevertheless, the optimum dosage of Seri Serdang seeds extracted by 1 mol L^{-1} NaCl solution is two times lower than that of the Serdang Raya seeds which are extracted by the same NaCl solution. The optimum dosage for the Seri-Serdang seed was 40 mg L^{-1} and 80 mg L^{-1} for the Serdang-Raya. It should be mentioned that the turbidity removal in both sources of seeds was almost similar, 97% for the Seri-Serdang seeds and 96.5% for the Serdang-Raya.

As the study results indicate, the levels of turbidity removal using Seri Serdang seeds as a proper source by different extraction methods were quite similar under the different experimental conditions and the differences observed between these treatments were insignificant. However, the main differences between the treatments were the amounts or concentrations of the optimum dosages. Shell-free *Moringa oleifera* seeds extracted by 1.0 mol L^{-1} NaCl solution showed a better coagulation activity with dosages 3.5 times lower than water extraction seeds for the removal of an initial kaolin turbidity of 200 NTU. Overdosing stands as another potential explanation for these differences. For example, Muyibi and Evision [25] have reported that overdosing results in the polymer bridge sites becoming oversaturated and this has the immediate effect of re-stabilizing those already destabilized particles due to lack of an adequate number of particles that may form inter-particle bridges. Okuda et al. [2] suggests improving the coagulation effect of the *Moringa* seeds by extraction with salt. The enhanced coagulation efficiency by the salt extracts is due to the fact that (i) protein solubility in the salt is higher than that of the distilled water and (ii) the active components in both extracts mainly includes proteins; salting-in enhances the protein-protein dissociation by addition of salt(s) not common with those already available in the suspension [2].

Moreover, the differences in coagulation efficiency between the two sources is supported by the findings of Narasiah *et al*. [26] who demonstrated that different origins (geographic locations) of the *Moringa* seeds correspond to varying coagulation efficiencies which can be attributed to differences in the protein content of the seeds. On the other hand, this finding conforms to progressive genomics research worldwide which generally shows that plants synthesize different, yet closely related, proteins during their various stages of development [27].

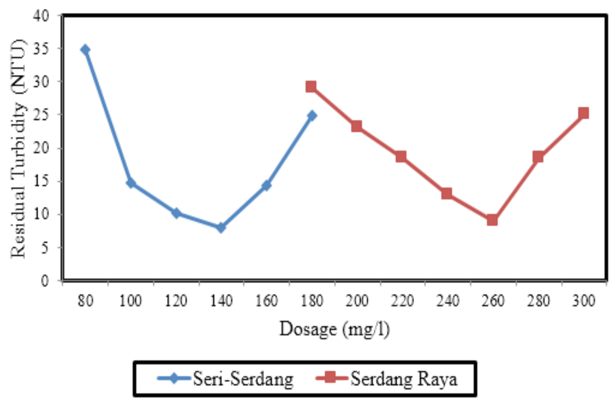

Fig. 1. Optimum dosage of DW extracts of shelled *Moringa* seeds derived from Serdang Raya and Seri-Serdang for an initial suspension turbidity of 200 ± 5 NTU.

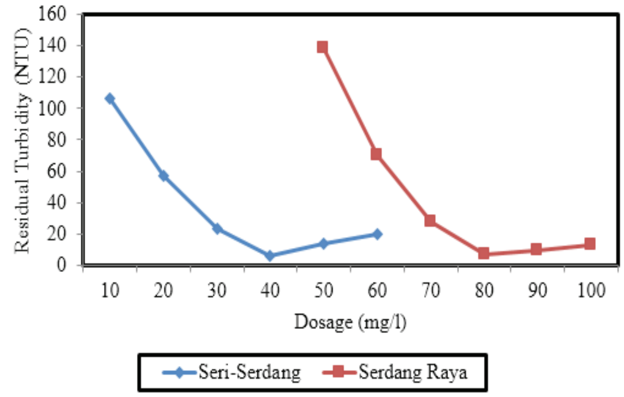

Fig. 2. Optimum dosage of NaCl extracts of shelled *Moringa* seeds derived from Serdang Raya and Seri-Serdang for an initial suspension turbidity of 200 ± 5 NTU.

3.2. Protein evaluation

Numerous studies have documented the main water and salt extractable components of the *Moringa oleifera* as proteinaceous [2,4,28]. Ghebremichael et al. [28] have conducted purification and characterization of salt and water *Moringa oleifera* seed extracts. They documented that the active agents in the two types of extracts are cationic proteins exhibiting isoelectric points above 9.6 and molecular masses lower than 6.5 kDa. The studies have shown that the active ingredient of Moringa oleifera seeds includes a minimum of four homologous proteins which do not dena-ture after five hours of heating at 95 ^{o}C [28].

Protein concentrations for all the *Moringa oleifera* Seri-Serdang and *Moringa oleifera* Serdang-Raya seeds were determined by the dye-binding method [23] and presented in Table 2. The obtained results confirmed that the proteins are the main active ingredient in the Moringa oleifera seeds which is also in agreement with the results of all previous studies [2,4,28]. On the other hand, these results showed that the varying coagulation efficiencies between different sources of seeds native to Serdang-Raya and Seri-Serdang could be attributed to the different protein contents of various seeds where the amount of soluble protein in crude water extract of the shelled Seri-Serdang seeds was almost two times greater than the corresponding amount in crude water extract of the shelled Serdang-Raya seeds. Therefore, the seeds from Seri-Serdang were chosen for further investigation of the coagulation effects of the *Moringa oleifera* in this work.

Table 2. Protein concentration of different samples of *Moringa oleifera.*

Samples	**Protein (mg BSA L^{-1})**
Water extract of shelled seeds (Seri-Serdang)	815
Water extract of shelled seeds (Serdang-Raya)	437.5
NaCl extract of shelled seeds (Seri-Serdang)	2850

3.3. Influence of pH

Table 3 illustrates the effects of different pH values of the water samples on turbidity removal by shelled *Moringa* seeds extracted by 1 mol L^{-1} NaCl. The pH range under examination was 5 to 9. This particular pH range was adopted as it complies with the common pH values of most water sources. The results indicated that the residual turbidity does not change significantly and remains almost constant during all the considered pH values (varied between 5.7 NTU and 8.2 NTU). The differences between this research work and one conducted by Nabigengesere and Narasiah [9] is related to the extraction methods used. Ndabigengesere and Narasiah, [9] reported that the coagulation of the shelled *Moringa oleifera* seeds extracted by the distilled water was independent of the pH values of the water sample. However, interpretation of the role that suspension pH plays in the removal of turbidity can be done only if an isoelectric pH is earmarked both for the coagulant (e.g., *Moringa*'s proteins) and the de-stabilizer (e.g., kaolin,). Isoelectric points for the kaolin and the *Moringa oleifera* were found as 2.8 and 10, respectively. This means that the *Moringa*'s cationic proteins assume and maintain a net positive charge as long as the pH is below 10 while kaolin particles take a net positive charge at pH values greater than 2.8. As a result and in light of the postulation that charge neutralization and adsorption are the key processes governing coagulation by the *Moringa oleifera*, proteins and kaolin surfaces were dominated by positive and negative charges within the examined pH range [9], respectively.

3.4. Alkalinity, hardness and salinity

Tables 4 through 7 summarize the effects of different concentrations of HCO_3^- alkalinity, and hardness, and salinity separately on turbidity removal by the shelled *Moringa oleifera* seeds which are extracted with NaCl. The results have been derived from coagulation experiments whereby different concentrations of alkalinity, hardness, and salinity were separately applied to the synthetic turbid water. The concentrations of alkalinity, hardness, and salinity were varied in such a way as to mimic corresponding possible values in an actual water sample. This approach indicates that the surface water and wastewater invariably have different concentrations of major and minor anions and cations; accordingly, examination of the coagulation efficiency potential and capacity of *Moringa oleifera* and its capability to remove turbidity just by employing kaolin-distilled water suspensions will not express the *Moringa*'s attendant performance in the real world. Therefore, no generalizations could be made.

Table 3. Effect of pH on turbidity removal by coagulation with *Moringa Oleifera* seeds extracted with NaCl (Initial Turbidity 200NTU)

No	pH	Turbidity removal (%)
1	5	96.85
2	5.5	97.15
3	6	97.05
4	6.5	96.6
5	7	96.25
6	7.5	95.9
7	8	96.35
8	9	96.5

Ndabigengesere and Narasiah [9] have studied the effects of different concentrations of anions and cations on turbidity removal by the shelled *Moringa* seeds extract using distilled water. They reported that these parameters did not have significant effects on turbidity removal by the *Moringa oleifera*. Since the studies which investigate these effects are quite common in the published literature, there is not much work using the shelled *Moringa* seeds extract by 1 mol L^{-1} NaCl. The current study contributes to the exploration of new perspectives for maximizing the efficient use of the *Moringa* seeds in water treatment through investigating the efficiency of the turbidity removal by the shelled *Moringa* seeds extract using 1 mol L^{-1} NaCl, in comparison with the technique which uses distilled water [9].

It can be inferred from Tables 3 through 6 that the different amounts of alkalinity (HCO_3^-), hardness (Ca^{2+}, Mg^{2+}) and salinity (NaCl) have no significant effect on turbidity removal by the *Moringa oleifera* seeds which are extracted with NaCl. The turbidity removal affected by alkalinity was obtained between 92.35% (25 mg HCO_3^-/ l) and 94.7% (250 mg HCO_3^-/ l), calcium hardness between 95% (25 mg Ca^{2+}/l) and 93.8% (250 mg Ca^{2+}/l), and magnesium hardness between 95.3% (25 mg Mg^{2+}/l) and 91.2% (250 mg Mg^{2+}/l). As to salinity, the turbidity removal was reported ings of Ndabigengesere and Narasiah [9] which showe0d that the concentrations of anions and cations (Mg^{2+}, Ca^{2+}, HCO_3^- and NaCl) have no significant effects on the turbidity removal by the *Moringa oleifera*. Since it is already well-established that the destabilization of the kaolin particles is the main coagulation mechanism involved in the case of *Moringa*, the presence of the anions and cations are critical to the success of the coagulation process [9]. However, it may be said that speculation on the potential of coagulation of kaolin-distilled water suspensions are of no practical significance because all surface water and wastewater differ markedly in terms of constituents and constituent concentration; they are more often than not mineralized to a greater or lesser degree. Nevertheless, the current research provides evidence that the shelled *Moringa* seeds extracted by 1 mol L^{-1} NaCl is a coagulant suitable for any turbid water as far as different amounts of alkalinity, hardness, and salinity are concerned.

Table 4. Effect of Alkalinity on coagulative turbidity removal with *Moringa oleifera* seeds extracted by NaCl (Initial Turbidity 200NTU)

No	(mg L^{-1})	Turbidity removal (%)
1	25	92.35
2	50	94.3
3	75	93.5
4	100	92.95
5	150	93.6
6	200	93.9
7	250	94.65

Table 5. Effect of Ca^{2+} on coagulative turbidity removal with *Moringa oleifera* seeds extracted by NaCl (Initial Turbidity 200NTU)

No	Ca^{2+} (mg L^{-1})	Turbidity removal (%)
1	25	95
2	50	93.25
3	75	92.1
4	100	91.8
5	150	90.5
6	200	91.95
7	250	93.75

Table 6. Effect of Mg^{2+} on coagulative turbidity removal with *Moringa oleifera* seeds extracted by NaCl (Initial Turbidity 200NTU)

No	Mg^{2+} (mg L^{-1})	Turbidity removal (%)
1	25	95.25
2	50	93.95
3	75	92.85
4	100	92.1
5	150	91.5

Table 7. Effect of NaCl on coagulative turbidity removal with *Moringa oleifera* seeds extracted by NaCl (Initial Turbidity 200NTU)

No	NaCl ($mg\ L^{-1}$)	Turbidity removal (%)
1	25	96.65
2	50	96.45
3	75	96
4	100	95.15
5	150	95.5
6	200	94.3
7	250	93.75

4. Conclusion

Protein content analysis by the dye-binding method disclosed, and hence affirmed, that Moringa seeds of different sources had different coagulation efficiencies which mostly relate to the differences in their protein contents. Furthermore, the amount of soluble protein in the crude salt extract of the Moringa seeds had a considerable amount compared to the respective water extract of the Moringa seeds.

The results of our experiments performed on shell-free *Moringa oleifera* seeds extracted by NaCl solution showed better coagulation activity than those extracted using distilled water, where the former had an optimum dosage of 40 mg L^{-1} and brought about 97% turbidity removal. On the other hand, the crude salt extract of *Moringa oleifera* shell-free seeds exhibited turbidity removal that was superior to the corresponding turbidity removal with the distilled water extract. The former had a dosage which was 3.5 times lower than that of the latter.

The projections and generalizations from previous studies concerning the potential coagulative performance of the *Moringa* seeds on synthetic wastewater suspensions do not reveal any practical significance because the water or wastewater to be treated will undoubtedly and invariably differ in composition, in terms of component quantity and quality, from the synthetic wastewater.

Complementing previous studies, the results of this study provided some evidence that the concentration of a number of water quality parameters of the target water, like salinity, alkalinity, and hardness, have no significant effect on the coagulative turbidity removal of the *Moringa oleifera* seeds. In conclusion, this study demonstrates that the seeds of *Moringa oleifera* seem to be a promising coagulant that performs appreciably well with turbid water and wastewater irrespective of their quality including the type of constituents and respective concentrations.

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