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ORIGINAL ARTICLE

Formulation of Hydrogel Containing *Oenanthe Javanica* Extract for Topical Use: Extraction, Formulation and Characterization.

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Abstract
Human skin is exposed to aging factors such as sun exposure and environmental pollutants, which cause unwanted stress on the skin, primarily due to the generation of reactive oxygen species (ROS) within the skin. Antioxidants can slow down, prevent or reverse aging by neutralizing the free radicals produced by ROS. *Oenanthe Javanica (O. javanica)* extract carries a potential in the treatment of skin aging due to its high antioxidants content. *O. javanica* extract (0.8%) was incorporated into a hydrogel carrier at different formulation compositions (A1, A2, A3 and A4). Throughout a 30-day stability study, all formulations were found to maintain optimum pH value within the range of 4.5 to 5.5. No significant changes in the physical appearance of the hydrogel containing extract were observed. Sufficient preservative was added to the hydrogel, and no microbial growth was observed on nutrient agar plates after storage under optimal conditions for microbial growth. As for the smoothness evaluation, 9 out of 10 volunteers preferred and were most satisfied with A4 formulation. All formulations were found to be denser than water, ranging between 24.24 and 26.94. Significantly, the percentage of moisture loss was found to be between 89.77 to 90.33%, indicating all the formulated hydrogels contained high amount of water, providing the moisturizing effect. Rheological analysis demonstrated that the hydrogel exhibited pseudoplastic and shear-thinning behaviour. Thus, the novel formulated hydrogels containing *O. javanica* extract could open up new possibilities for the production of cosmeceutical products.

Keywords: *Formulation, Hydrogel, Oenanthe Javanica, Topical, Anti-aging*

Introduction

Human skin is exposed to aging factors such as sun exposure and environmental pollutants, which cause unwanted stress on the skin. According to McDaniel e*t al.* (2017), skin exposed to UV radiation can generate reactive oxygen species (ROS) within the skin [1]. ROS led to the oxidation of DNA, resulting in photodamage and visible tissue aging. Furthermore, Tobin (2017) claimed that ROS can instigate gene expression pathways that lead to increased deterioration of collagen and accumulation of elastin [2]. Excessive quantities of ROS, referred to as oxidative stress, lead to modifications in cell components [3]. Poljšak *et al.* (2011) stated that oxidative stress is the imbalance between the activity of antioxidant defenses and the production of ROS [4].

López-Alarcon and Denicola (2013) reported that antioxidants have the ability to slow down, prevent or reverse aging by neutralizing the free radicals produced by ROS [5]. Although antioxidant do not eliminate all oxidants, they can help maintain the oxygen homeostasis [6]. Antioxidants act as reducing agents that assist in the deactivation of ROS. *Oenanthe javanica (O. javanica)* extract shows potential in the treatment of skin aging due to its high content of antioxidants components. According to Bhaigyabati *et al.* (2017), *O. javanica* contain abundance of phenolic compounds that possess natural antioxidant properties [7]. Phenolic compounds possess antioxidant activity by donating hydrogen atoms to free radicals, which can reduced the oxidative stress [7]. The 2,2-diphenyl-1-picryl- hydrazyl-hydrate (DPPH) free radical scavenging assay showed that *O. javanica* has potent antioxidant properties due to the presence of high levels of phytoconstituents such as phenols and flavonoids [8]. Methanol extraction yields a higher phenolic and flavonoid content than the water extract method [7].

Hydrogel is an organic polymeric gel commonly combined with more complex drug-delivery systems and has a moisturizing effect. Hydrogels are also more stable under changes in temperature and pH compared to emulsion and cream [9]. Hydrogel containing antioxidants can hydrate the skin, restore its elasticity, and promote anti-aging effects. Hydrogel has a low primary irritation index (PII) since it is primarily used in cell culture and biomedical applications that require low irritation index. PII is crucial to assess in cosmetics for skin and eye safety. Cosmetic-based hydrogel products have been shown to help with skin hydration, elasticity restoration, and promote anti-aging effects. According to Silna *et al.* (2016), hydrogels are suitable as topical moisturizers because they have a low irritation index for both eyes and skin [10].

Brady *et al.* (2017) stated that hydroxypropyl methylcellulose (HPMC) is made up partly from O-methylated and O-(2-hydroxypropylated) cellulose ether derivative polymers [11]. HPMC is one of the polymers used in formulating hydrophilic drugs, and is a biodegradable, biocompatible polymer that can be widely used in drug delivery, cosmetics, adhesives, dyes and many other fields [12]. It can also absorb and hold moisture, which is beneficial in cosmetic formulation [13].

Most antioxidants are being administered orally. Oral antioxidant supplements need to undergo first-pass metabolism, which reduces their bioavailability due to rapid metabolism in the liver and intestines. Regular consumption of antioxidant supplements can cause many unwanted side effects. Bhaigyabati *et al.* (2017) noted that prolonged and regular consumption of oral nutritional supplements, such as vitamin E and multivitamins, is associated with a higher risk of prostate and lung cancer [7]. Oral administration of antioxidant supplements such as beta-carotene, vitamin E, C , and A can increase risk of mortality with regular high dose consumptions [14]. Therefore, the formulation of hydrogel containing *O. javanica* extract, which is rich in antioxidant compounds, is believed to be beneficial for the topical application in addressing skin aging and opens up new possibilities for the production of cosmeceutical products.

Materials and methods

Materials

Oenatha Javanica (*O. javanica*) was purchased from local market in Malaysia. Xanthan gum and phenonip were purchased from Esson Haus Sdn Bhd (Malaysia). Carbopol, HPMC, glycerine, potassium sorbate and sodium benzoate were purchased from Personal Formula Resources (Malaysia) Sdn Bhd.

Extraction of O. javanica plant

Following the method of Akkol *et al.* (2012) with some modifications, a liquid-liquid extraction technique was employed for the extraction of *O. javanica* [15]. Three kilograms of *O. javanica* leaves were collected and dried for 3 days at 40°C temperature. The leaves were then ground and macerated in methanol at ratio weight of 1:10 (*O. javanica*: methanol) and shake for 3 days. The mixture was filtered using a vacuum pump. Then, the solvent was removed from the mixture using rotary evaporator at 40°C under a pressure of 100 mbar. The plant extract was placed in a centrifugal tube and freeze dried for 4 days.

Solubility study

A 0.1% of plant crude extract was dissolved in water medium. The mixture was then stirred and heated (if necessary) to obtain a clear solution. Once the clear transparent solution was obtained, an additional 0.1% of plant crude extract was added into the same mixture. Similar observations were made on the solution's appearance until a cloudy solution was obtained, at which point the cycles were stopped. The maximum amount of plant crude extract that successfully dissolved in the fixed water medium was recorded.

Formulation of hydrogen loaded with O. javanica extract

The hydrogel was formulated by mixing xanthan gum, HPMC, carbopol and 10 mL of distilled water. The mixture was heated and stirred at 60°C until all ingredients were dissolved. Glycerin, potassium sorbate, and sodium benzoate were then added into the mixture. After that, the *O. javanica* extract was added into the vehicle mixture, which was topped up with distilled water until 100 g of hydrogel was achieved. The formulated hydrogel was further homogenized using an overhead homogenizer (Daihan Scientific UR Partner in Laboratory, Korea) at 200 rpm for 1 hour. Drops of phenonip was added to the hydrogel 10 mins before the process completed. Four formulations (A1, A2, A3 and A4) were formulated at different compositions as shown in Table 1 [16].

pH evaluation study

The pH of A1, A2, A3 and A4 were determined using an electrode digital pH meter (Eutech Instruments Pte. Ltd.). pH meter was first calibrated using different pH buffer (pH 4, 7 and 10) to ensure proper functioning. This study was carried out weekly for one month period in triplicate. All data were recorded and analysed [17].

Accelerated stability study

The formulated hydrogels (A1, A2, A3 and A4) were placed in 15 mL centrifugal tubes and subjected to centrifugation at 2,300 rpm for 15 mins. Evaluation on the stability of hydrogels was carried out with respect to their physical appearance, and any noticeable observations were recorded.

Microbial growth study

Nutrient agar was used for the microbial growth study. The hydrogels sample were aseptically transferred onto the sample plates in a cross pattern. The microbial growth was observed daily for 14 days [16].

Smoothness study

Surveys were filled up by 10 volunteers to avoid biased results. All the formulated hydrogels were applied on volunteers' skin and their comments with respect to appearance, smoothness, absorbance ability, oily feeling, itchiness and sign of irritation were recorded using the constructed questionnaire [16].

Relative density study

The weight of empty pycnometers with stopper was measured and recorded. Then, the pycnometer was filled with distilled water until it overflowed and the stopper was placed. The weight of the pycnometer containing distilled water was re-weighed and recorded. The same procedure was repeated for different formulations of hydrogels [16]. Analysis was carried out in triplicate for each sample and the obtained data was analysed using the Equation 1.

$$\text{Relative density of sample w.r.t water} \qquad \text{(Equation 1)}$$

$$= \frac{\text{Density of sample}}{\text{Density of water}}$$

$$= \frac{\text{mass of sample}}{\text{mass of water}}$$

Moisture content evaluation study

Petri dish weight was first taken and recorded. A 3 g of the formulated hydrogel was placed in the respective petri dish and kept in a desiccator containing anhydrous calcium chloride. After three days, the formulations were re-weighed, and readings were recorded [16]. This study was carried out in triplicate for each sample. The percentage moisture loss was calculated using Equation 2.

$$\text{Percentage moisture loss} = \frac{\text{(Initial weight} - \text{Final weight)}}{\text{Initial weight}} \times 100\% \qquad \text{(Equation 2)}$$

Rheology study

A Modular Compact Rheometer (Anton Paar, USA) was used to determine the rheological behaviours of the hydrogels (A1, A2, A3 and A4). The shear rate was ranged from 0.01 to 50.00 sec^{-1} at a controlled temperature (25°C). Cone and plate geometry of 4^0/40 mm was used, and the plate gap was set at 0.15 mm. After the sample was placed on the plate, it took 5 mins for the instrument to equilibrate the measurement beforehand. From the 25 data points generated, average viscosity results were recorded in pascal second (Pa·s). The formulated hydrogels were analysed with respect to its behaviour which fit the power law model [18].

Statistical analysis

All analyses were carried out in triplicate and all data are shown as mean ± standard deviation (n=3).

Results and discussion

Extraction of O. javanica

The plant extract of *O. javanica* leaves, obtained through the maceration technique with methanol, resulted in a 22.05% yield of extract. Methanol was chosen because it has been reported to be the best solvent to yield highest amount of phytoconstituents compared to water [19]. Methanol has a methyl group that forms more bonds with the constituents of *O. javanica*, whereas water, with only two hydrogen and one oxygen atom, forms fewer bonds with the extract constituents. Truong et al. (2019) also stated that, methanolic extract produced the most potent extract with the highest radical scavenging activity, indicating higher antioxidant activity [19]. The appearance of the crude extract was dark green and sticky. This is due to the extraction using methanol that can extract sucrose, glucose and other sugars [20]. Quispe-Condori *et al*. (2011) also reported that the sugars do not evaporate during freeze drying and are left as a sticky mass [20]. If the sugar content is very low, powder form can be achieved after freeze drying.

Solubility test of O. javanica extract

Distilled water was observed to successfully solubilized the *O. javanica* extract up to 0.8%

(Table 2). One of the essential criteria for achieving the desired concentration of the drug for pharmacological response is solubility, which involves the dissolution of the solvent to give a homogenous system [21]. Savjani *et al.* (2012) stated that the degree of solubility of a product in a particular solvent is assessed as the concentration of saturation, beyond which no further increase occurs [21]. This statement supports the solubility of *O. javanica* extract in water, as the concentration stop increasing at 0.8%.

pH stability analysis

The average pH reading of the formulated hydrogels (A1, A2, A3 and A4) throughout 1 month storage at 4^0C were recorded (Table 3). All hydrogels were observed to maintain a steady pH throughout the one month observation period, with stable pH values found (Figure 12). Hydrogel of A3 and A4 showed the most stable and consistent pH readings throughout the 1 month storage. Even though A1 and A2 showed a slight increment on the pH values, the changes were not significant, with less than 5% variation. Tracking pH values is vital in determining the stability of hydrogels. Any changes in pH value indicate the presence of chemical reactions which could affect the final product's quality. Some *et al*. (2000) claimed that the most significant components of chemical stability are accelerated testing performance and pH profile kinetics [22]. The pH of human skin generally varies between 4.5 and 6.0. In order to be suitable for industrial application, the pH of a formulation need to be in this range [23].

Hence, it was demonstrated that all the formulated hydrogels possessed a suitable pH range as intended by the industry. Hydrogel A3 exhibited the best property in this analysis, showing stable pH values throughout the 1-month period. It also had the most suitable pH values for human skin application, which can help avoid skin irritation.

Microbial growth analysis

Based on the observation (Table 4), no microbial growth was present on the agar plate for A1, A2, A3 and A4 after storage in an incubator at 38^0C for 14 days. Zhang *et al.* (2017) claimed that the presence of microbial growth, with colony count, is crucial for determining whether the preservative (phenonip) used in this study is effective in inhibiting the growth of microorganisms [24].

Smoothness analysis

The summary of feedback from 10 volunteers by rating on all the formulated hydrogels (A1, A2, A3 and A4) were recorded (Table 5). Majority of volunteers (90%) prefer hydrogel A4 and A1 which provided a comfortable feeling and fast absorption. Fifty-one percent of the volunteers stated that hydrogel A2 and A3 took time to absorb and were a little bit sticky, indicating slower absorption of the product. Eighty percent of the participants strongly agree with the appearance of hydrogel A4, while the least agreement was for hydrogel A1 with only 30% of the participants strongly agreeing. All participants (100%) preferred the smoothness of hydrogel A4, while only 60% preferred hydrogel A2. All participants (100%) strongly agreed that none of the hydrogels irritated the skin. Additionally, 100% of the participants strongly agreed that hydrogels A1, A2 and A4 did not cause any itchiness, while hydrogel A3 had only 90%. One hundred percent of the participants strongly agreed that hydrogel A4 did not have an oily characteristic, while 80% of the participants strongly agreed that hydrogel A2 for the same evaluation.

Hydrogel A2 contains a high amount of HPMC, while hydrogel A3 contains a high amount of xanthan gum . Soni et al. (2018) stated that, high concentration of xanthan gum and HPMC can exhibit an unpleasant sticky feeling on the skin [25]. HPMC has high bioadhesion characteristics, which result in stickiness on the skin and make it more suitable for in vivo formulations [26].

Hydrogel A4 contain carbopol as the main polymer in the formulation, as Soni et al. (2018) stated that carbopol will remain pleasant even at high concentration [25]. Hence, this supports hydrogel A4 to be the most preferred formulation for its transparent appearance and good consistency among the other formulations [27]. Hydrogel A3 contain a high amount of xanthan gum, which Brunchi *et al*. (2016) stated gives the formulation a cloudy appearance and sticky consistency that can create bubbles [28]. Some of the volunteers found that hydrogel A3 tended to be slightly itchy on the skin, which Kovács *et al.* (2020) mentioned as a common effect of xanthan gum on broken skin barrier, causing an itchy sensation [29].

Krongrawa *et al*. (2018) stated that hydrogel is a water-based formulation that should be oil-free, which makes it light and have a watery texture [30]. This is supported by the positive feedback from the volunteers. The participants ranked the formulated hydrogel from the most liked to most disliked hydrogels as follows: A4 > A1 > A2 > A3. This shows hydrogel A4 has the best properties, being preferred by most of the volunteers.

Relative density analysis

The relative density measurements of samples were recorded to be in the range of 24.24 to 26.94 (Table 6). The formulated hydrogels (A1, A2, A3, and A4) were found to be denser than water due to the high concentration of excipients and active ingredients forming hydrogen bonds with water [31]. Hydrogel A2 had the highest relative density of 25.94. The degree of ionization of the polymers HPMC, carbopol and xanthan gum affects the density of ionically crosslinked membranes, making the different polymers to have different densities [32]. Thus, the ingredients in the formulation could influence the relative density of the hydrogels. Hydrogel A2 shows the best property in this analysis, having the highest density among the other hydrogels.

Moisture content analysis

It was found that the percentage of moisture loss in all formulated hydrogels ranged from 89.77 to 90.33% (Table 7). Hydrogel A3 had the highest moisture content at 90.33%, followed by A2 (90.22%), while A1 and A4 had the same moisture content (89.77%). All the hydrogels showed a high percentage of moisture content, indicating a high concentration of water . Goh *et al.* (2019) stated that a high percentage of moisture content produces a good hydrating effect, characterized by a significant increase in skin moisture and smoothness [33]. High moisture content can also repair the skin barrier function and provide moisturization without causing irritation [34]. Hydrogel A3 has the best properties in this analysis, with the highest moisture content, which will increase skin moisture and smoothness. It was also found to repair the skin barrier function and be non-irritating. All hydrogels exhibited a moisture content percentage of more than 80%, which is a good indicator of their effectiveness in moisturizing the skin.

Rheology analysis

The viscosity of the formulated hydrogels was found to range between 78.81 Pa.s and 186.23 Pa.s (Figure 2). Hydrogel A4 exhibited the highest viscosity level of 186.23 Pa.s. Pounikar *et al.* (2012) stated that the higher viscosity provides greater thickness and consistency to the gel [35]. The high viscosity of hydrogel A4 is attributed by its high ratio of carbopol content [35]. All the hydrogels displayed a flow curve with a gradual loss of viscosity as the shear rate increased (Figure 2b), indicating pseudoplastic and shear thinning behaviour [36]. The rheogram curve for pseudoplastic materials results from the shearing action on long-chain molecules, such as linear polymers. As shear stress increases, the disarranged molecules begin to align their long axes in the direction of flow, reducing the size of dispersed molecules and decreasing the apparent viscosity [37].

Figure 2a indicated that all the formulated hydrogels exhibited non-Newtonion behaviour. Non-newtonion behaviour is defined as a change in the viscosity of a fluid when shear is applied. Chhabra (2010) stated that the degree of the curve for shear stress against shear rate may vary due to several factors, such as the nature and concentration of the polymer, the nature of the solvent, and other parameters like particle size, shape, and polymer solutions [36].

Conclusions

Four different hydrogels (A1, A2, A3 and A4) containing 0.8% of *O. javanica* extract with different compositions were successfully formulated and analysed for their physicochemical and rheological characteristics. The pH values of the hydrogels were successfully maintained within the optimal range of 4.5 to 5.5 throughout the stability observation period of one month. Hydrogel A3 showed the best stability based on pH value analysis. No microbial growth was observed on the agar plates after been incubated at 38^0C for 14 days, indicating that all the formulated hydrogels were well-preserved with sufficient preservative content. In a transparency and smoothness study hydrogel A4 was preferred. In the relative density study, all the hydrogels were found to be denser than water, with similar relative density ranging between 24.24 and 26.94. The percentage of moisture loss in the hydrogels were found to be 89.77 to 90.33%, indicating all the hydrogel had a high moisture content with more than 80%, which is expected to provide excellent moisturizing effect. Lastly, rheological analysis confirmed that the hydrogels possessed pseudoplastic, shear-thinning and non-Newtonion behaviour. In near future, these studies should be extended to include prolonged stability analysis, with particular attention to the plant crude extract content throughout the storage. This will further support and validate the potential of the formulated hydrogel containing *O. javanica* extract as an anti-aging skincare product in the cosmeceutical industry.

Conflicts of interest

The authors have declared that no competing interests exist.

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Authors' Contributions

SHM: Conceived the research, conducted the analysis, and drafted the manuscript.
NKHKH: Study the theory, performed the analysis, drafted the results.
CG: Expert in plant extraction and verification.
All authors discussed the results and contributed to the final manuscript.

Table 1. Formulation's composition of A1, A2, A3 and A4.

Ingredients	Formulation composition (g, w/w)			
	A1	**A2**	**A3**	**A4**
O. javanica extract	75	75	75	75
Xanthan gum	0.5	0.375	0.75	0.375
HPMC	0.5	0.75	0.375	0.375
Carbopol	0.5	0.375	0.375	0.75
Glycerine	5	5	5	5
Potassium sorbate	0.5	0.5	0.5	0.5
Sodium benzoate	0.5	0.5	0.5	0.5
Distilled water up to	100	100	100	100

Table 2. The limit of *O. javanica* extract solubility in distilled water.

	***O. javanica* extract (%)**								
	0.1	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**
Solubility in distilled water	/	/	/	/	/	/	/	/	X

/ = soluble; X = not soluble

Table 3. The average pH reading for A1, A2, A3 and A4 for 4 weeks storage at 4^0C in triplicate.

Hydrogel formulation	Storage period (week)			
	1	**2**	**3**	**4**
A1	4.89±0.036	4.98±0.006	5.03±0.036	5.09±0.015
A2	4.87±0.006	4.98±0.006	5.03±0.036	5.07±0.156
A3	5.02±0.006	5.02±0.006	5.04±0.006	4.98±0.006
A4	4.74±0.025	4.88±0.025	4.87±0.035	4.87±0.035

Table 4. Colony count results for A1, A2, A3 and A4 after incubation at 38^0C for 14 days.

Hydrogel formulation	Storage period (week)			
	1	**2**	**3**	**4**
A1	X	X	X	X
A2	X	X	X	X
A3	X	X	X	X
A4	X	X	X	X

X = No colony formation

Table 5. Feedback from 10 volunteers by rating on the formulated hydrogel; (a) A1, (b) A2, (c) A3 and (d) A4.

(a)

Characteristics	Strength level				
	1	**2**	**3**	**4**	**5**
*Appearance				7	3
*Smoothness			1	2	7
*Absorbance			1	2	7
**Oily	10				
**Itchiness	10				
**Irritation	10				

*1- Poor, 2- Okay, 3- Moderate, 4- Good, 5- Very good

**1- Not at all, 2- Somewhat, 3- Quite a bit, 4- Very much, 5- Extremely

(b)

Characteristics	Strength level				
	1	**2**	**3**	**4**	**5**
*Appearance			2	2	6
*Smoothness				4	6
*Absorbance		1	2	4	3
**Oily	8	2			
**Itchiness	10				
**Irritation	10				

*1- Poor, 2- Okay, 3- Moderate, 4- Good, 5- Very good

**1- Not at all, 2- Somewhat, 3- Quite a bit, 4- Very much, 5- Extremely

(c)

Characteristics	**Strength level**				
	1	**2**	**3**	**4**	**5**
*Appearance		2	1	3	4
*Smoothness			1	2	7
*Absorbance	1	1	1	2	5
**Oily	8	2			
**Itchiness	9	1			
**Irritation	10				

*1- Poor, 2- Okay, 3- Moderate, 4- Good, 5- Very good

**1- Not at all, 2- Somewhat, 3- Quite a bit, 4- Very much, 5- Extremely

(d)

Characteristics	**Strength level**				
	1	**2**	**3**	**4**	**5**
*Appearance				2	8
*Smoothness					10
*Absorbance				1	9
**Oily	9			1	
**Itchiness	10				
**Irritation	10				

*1- Poor, 2- Okay, 3- Moderate, 4- Good, 5- Very good

**1- Not at all, 2- Somewhat, 3- Quite a bit, 4- Very much, 5- Extremely

Table 6. Average weight of hydrogels in 25 mL pycnometer.

Formulation	**Volume of sample (mL)**	**Weight of sample (g)**	**Relative density**
Distilled water	25	25.00±0.00	1.00
A1	25	24.24±0.07	24.24
A2	25	25.94±0.04	25.94
A3	25	24.48±0.02	24.28
A4	25	24.79±0.04	24.79

Table 7. Average weight loss of formulations in desiccator for 3 days.

Hydrogel	**Initial Weight (g)**	**Final Weight (g)**	Moisture content (%)
	3±0.000	0.307±0.006	89.77%
A2	3±0.000	0.293±0.006	90.22%
A3	3±0.000	0.29±0.000	90.33%
A4	3±0.000	0.307±0.006	89.77%

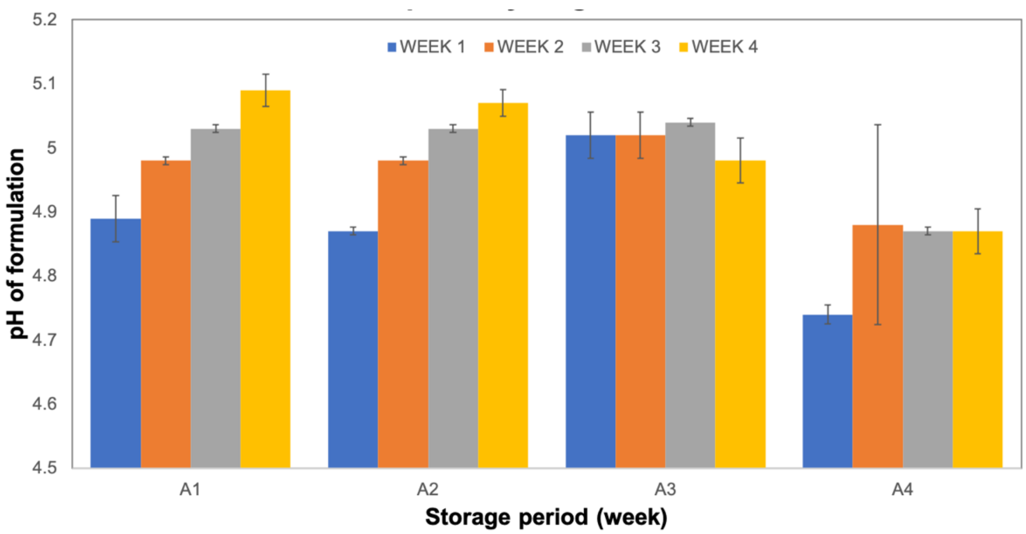
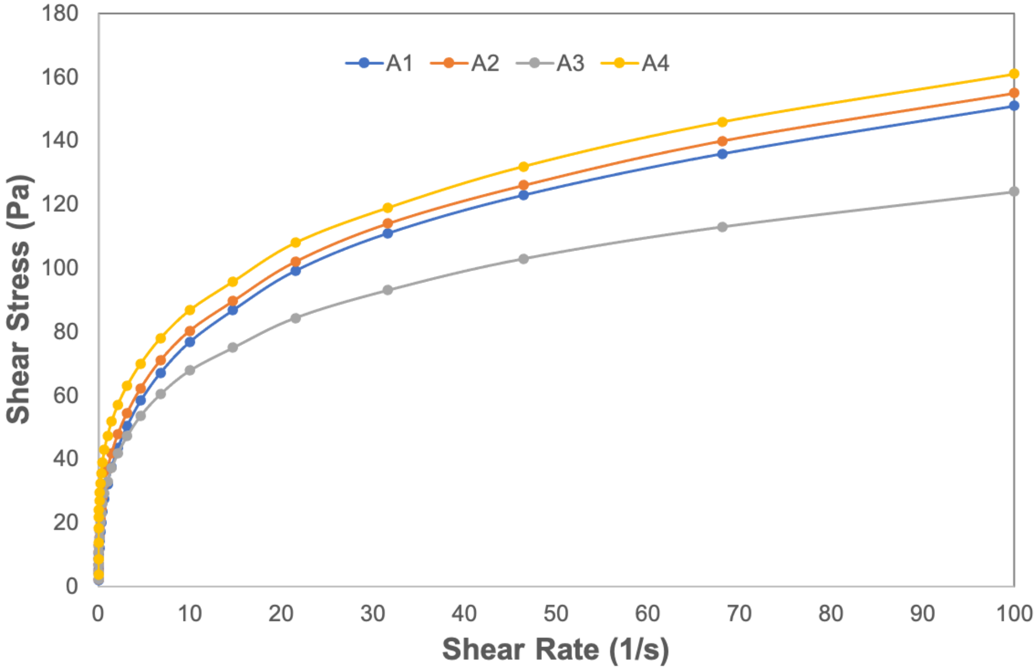


Figure 1. The pH value of A1, A2, A3 and A4 throughout 1 mth storage at 4^0C temperature.

(a)



(b)

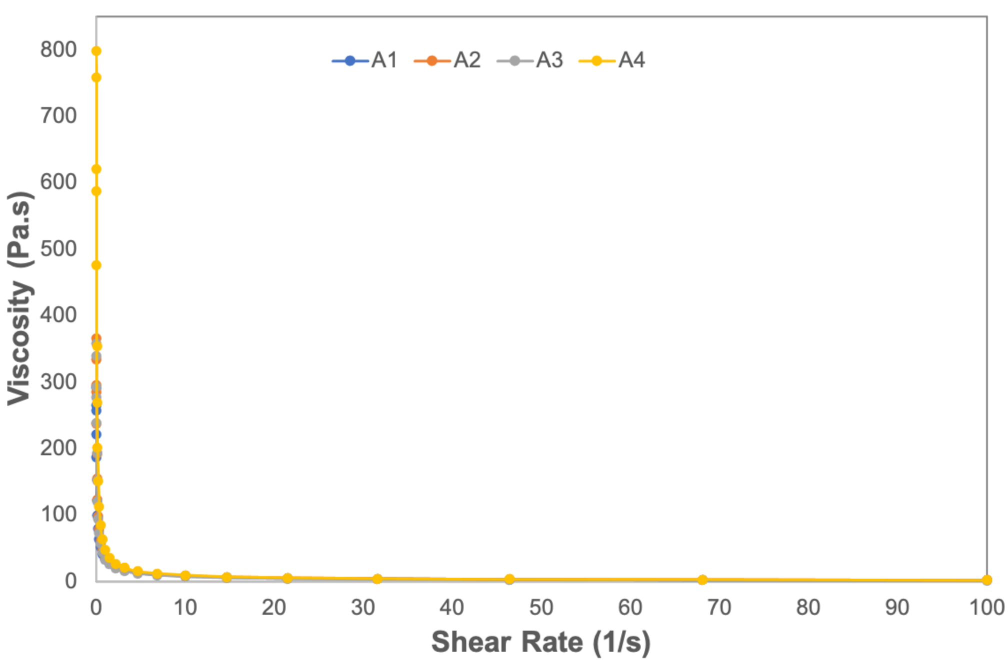


Figure 2. (a) Shear stress against shear rate; (b) Viscosity against shear rate.

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