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Impact of Starch Concentration on Chlorella-k-Carrageenan Gel Formation Mechanism

İrem Doğruoğlu[1], Yağmur Akarsu[1], Çağla Selen Şen[1], Onder Pekcan[1], Bengü Özuğur Uysal[1]

1 Kadir Has University

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Abstract

Despite their industrial popularity, plastics; specifically in packaging, have been known for their harmful environmental impact. In this work, as an alternative to plastic packaging, microalgae, and starch-based materials were employed. Here, gel formation mechanisms were investigated by using photometric analyses, namely photon transmission and absorbing techniques. Heating (gel-sol) and cooling (sol-gel) experiments were performed, and absorbance (A), transmittance (T), and Arrhenius plots were generated to observe the behavior of the mentioned materials during phase transition processes. The heating and cooling experiments revealed that the addition of starch quickened the gel-sol transitions but slowed down the sol-gel transitions. Hence, the results suggested that the sample containing *Chlorella, k-Carrageenan*, and starch was the most promising potential alternative for plastic packaging.

İrem Doğruoğlu[a], **Yağmur Akarsu**[a], **Çağla Selen Şen**[a], **Önder Pekcan**[a], and **Bengü Özuğur Uysal**[a,*]

[a]*Faculty of Engineering and Natural Sciences, Kadir Has University, Cibali, Fatih, Istanbul 34083, Turkiye*

[*]Corresponding author. E-mail: bozugur@khas.edu.tr

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1. Introduction

Microalgae are eukaryotic or prokaryotic organisms consisting of a single cell, living in soil, freshwater, and marine ecosystems. They can grow alone or as colonies in water ecosystems, especially lakes and rivers. They can produce bioactive metabolites through photosynthesizing. Due to their ability to grow rapidly, easy adapting capabilities to different cultivating environments, and wide utilization potential among many fields, research on microalgae has been on the increase [1]. Some examples include, but aren't limited to, pharmaceuticals, biofuels, bioplastics, and food products (such as supplements) [2]. In addition to that, they also have the potential to replace liquid fossil fuels, acting as a possible

solution to issues such as sustainability, price, and pollution. Among the researched microalgae, *Carrageenan* and *Chlorella* have also been evaluated as potential candidates [3].

Carrageenan is a natural gel-forming polysaccharide extracted from red seaweeds, containing an abundant amount of cellulose. It doesn't have any nutritional value and is often utilized in the food industry for its thickening, gel-forming, viscosity-boosting characteristics [4]. Other than the food industry, it's also been commonly used in fields such as cosmetics, engineering, pharmaceuticals, biotechnology, and many more [5].

The microalgae *Chlorella vulgaris* belongs to the order of *Chlorococcales of the Oocytaceae* family and the genus *Chlorella*. Due to the chloroplasts, it has a green color, and a high ability to perform photosynthesis. Other than chlorophyll, *Chlorella vulgaris* contains an abundance of proteins, lipids, carbohydrates, β-carotenes, minerals, pigments, and vitamins [6]. This makes *Chlorella vulgaris* a popular ingredient in food supplements, clinical treatments, cosmetics, and detoxifying wastewater [7].

Microalgae can be utilized in many sectors. Their nutritional value makes them a widely employed material, especially in the food industry. In addition to being a commonly known material in the food industry, they can also be utilized in making pigments and cosmetics [6]. Another very promising use of microalgae is as a source of biofuel. Since they are rich in lipid content and biomass productivity, they can take the place of chemical fertilizers as bio-stimulants. Apart from the previous uses of microalgae as mentioned, they are excellent sources for bioplastic production. Microalgae-derived bioplastics can be employed in a variety of industries, including but not limited to, transportation, medical hygiene, and food packaging. Due to their low toxicity, barrier properties, and biodegradability, bioplastics are preferred over petrochemical polymers [8].

The packaging industry has always prioritized the durability of packaging and enhancing product protection, without sufficient consideration for the material's disposability [9]. Numerous solutions to overcome disposability issues have been proposed, such as employing natural products.

In addition to microalgae, starch is the primary food resource of plants, and can be commercially produced from grains and tubers. It consists of two polysaccharides, amylose, and amylopectin [10]. Starch naturally occurs in starch granules, which are known to be highly organized structures. Many of the chemical and physical properties of starch originate from this granular structure. Due to starch's specific thermal characteristics and overall usefulness, it has been widely employed in industries such as food and packaging [11]. One specific characteristic of starch that allows it to be used in these industries is that when heated, starch granules break down into their polymers, causing gelatinization [12].

In this work, the aim was to create a microalgae-based alternative to plastic packaging and study whether starch would improve the structure of the material. Photometric analyses, specifically photon transmission and absorbing techniques, were used to evaluate gel formation mechanisms in this research. Additionally, to analyze the features of materials in phase transition processes, heating (gel-sol) and cooling (sol-gel) experiments were performed. The findings of these methods indicated that several of the materials utilized in these experiments could be an alternative to plastic packaging.

2. Methodology and Material

2.1. Gel Formation

Gel formation can be described as a spontaneous chemical reaction where a gel is formed, often through branched networks of linear flexible chains that get attached by covalent bonds [13]. Long polymer chains get cross-linked or associated into a three-dimensional network, immobilizing the liquid inside the solution, and forming a structure resistant to flowing [14]. In terms of solution composition or temperature, the process is externally alterable. It can be triggered by both physical and chemical factors, like heat, pressure, or the addition of ions. A sol-to-gel transition takes presence when gel formation occurs. The particles accumulate during the transition, resulting in a network that covers the entire container's volume [15].

Heat-induced gel formation is among the most used gel formation methods[15]. The first step is the detachment and unfolding of molecules post the initial energy input. The second step, following the unfolding, is the aggregation and association of these molecules to form the gel. To achieve these steps, the aqueous mixture containing a gelator is heated and then cooled [16].

2.2. Photon Transmission Technique

Photometric analyses can be conducted to assess the chemical properties of the gels, by determining their reactivity and light absorbances at certain wavelengths. Absorbance (A) is the measure of the light a solution is capable of absorbing [17]. Post-gel formation, these analyses were conducted to detect the sol-to-gel transition in detail, along with the reversible gel formation process transition [18].

A spectrometer is often used to measure the absorbance or transmission of light of a substance. Since the amount of light scattering gets affected by the presence of a gel network in a gel-forming solution, to detect the gel formation, a spectrometer can be used. With the help of photometric calculations, the pH, temperature, and concentration of gel-forming chemicals can be adjusted for ideal conditions [19].

An increase in absorbance or decrease in transmission can be seen through changing the parameters. This is because when the light passing through the gel is increased, the scattering of light increases as well. By applying these changes, gel formation dynamics and gel strength can be established [20].

2.3. *Chlorella*

Initially, only *Chlorella* and distilled water were used. All the samples were combined in 5 mL beakers which were purchased from ISOLAB. The *Chlorella* used in all samples came in powder form and was commercially obtained from the brand Kuru Yeşil. Table 1 can be seen below, and it provides information on the energy and nutrient values of the powdered *Chlorella*. The *Chlorella* content of all samples was weighed using a Series 390 Semi-Micro analytical balance

bought from the brand Precisa. In addition, the *Chlorella*-distilled water samples, several samples that contained different amounts of *Chlorella*, distilled water, and acetone bought from Sigma-Aldrich were all mixed. Magnetic stirrer bars bought from Fischer Scientific allowed the samples to be stirred properly. MTOPS MS300HS Hot Plate & Stirrer was used to stir and heat the mixtures to achieve the gel form.

Table 1. Energy and nutrients table for *Chlorella*.

Energy and Nutrients	***For 100 grams***
Energy	360 (kcal)
Total Fat	1.2 (g)
Saturated Fat	-
Trans Fat	-
Carbohydrate	36 (g)
Sugars	4 (g)
Fiber	0.4 (g)
Protein	64 (g)
Salt	-

2.4. Starch

In another set of trials, wheat starch was employed to obtain a better gel form. The wheat starch was obtained from a local market, from the brand Erdinç. The energy and nutrient values of the wheat starch used can be seen in Table 2 below. The method of preparing all the samples containing wheat starch was the same as described above.

Table 2. Energy and nutrients table for starch.

Energy and Nutrients	**For 100 grams**
Energy	295 (kcal)
Total Fat	0.1 (g)
Carbohydrate	73.1 (g)
Protein	0.25 (g)

2.5. *k-Carrageenan*

In an additional set of trials, *kappa-Carrageenan* (*k-Carrageenan)* was employed to test how it affected gel formation. The *k-Carrageenan* used in samples was bought from Sigma-Aldrich and came in powder form. The same products and methods were used as described in the *Chlorella* section.

After the trials*, Chlorella, k-Carrageenan,* wheat starch, and sodium chloride (NaCl) ended up being the materials used to achieve the gel-like form.

3. Experimental

3.1. Gel Formation

3.1.1. *Chlorella*-Water Gel Formation

In order to understand the chemical components of*Chlorella* and to examine the assumption that most organic microalgae materials can produce a gel by simply incorporating distilled water and heat, several ratios of *Chlorella* and distilled water (0.5:1, 1:1, 2:1) mixtures were prepared (Figure 1). *Chlorella* quantities were measured using an analytic balance and transferred to separate 5mL beakers for the addition of 5mL distilled water. When sample preparations were completed by placing a magnetic stirrer bar into each beaker, heating, and mixing were performed on a heating block for 15-20 minutes at 26-40 °C. Following that, samples were cooled at room temperature for about 5 minutes before being placed in an ice bath for another 5 minutes to promote gel formation.

Figure 1. *Chlorella* samples with distilled water in 1:1, 0.5:1, and 2:1 ratios (left to right) in 5mL beakers.

Following the first trials that included *Chlorella*, and distilled water, an experiment to improve gel formation efficiency was conducted by adding NaCl (0.06M) to samples separately since NaCl enhances the gel formation procedure through bond formation by cross-linking [21]. Afterward, samples were heated again in the same temperature range (50-80 °C) and were cooled at room temperature (25 °C). Therefore, proper gel formation was not observed. Additional trials using starch were performed to reach the gel formation stage since various biodegradable packaging manufacturers utilized starch and its texture properties regularly in their products [22].

3.1.2. *Chlorella*-Starch-Water Gel Formation

Since NaCl had already been detected as a promoter in gel formation in previous attempts, trials of integrating NaCl and starch to make the mixture more solid were done. Three different samples that contained several ratios of *Chlorella*,

starch, and NaCl were measured with analytic balance and collected in separate 5 mL beakers (Table 3). To observe the effect of starch on samples more clearly, the NaCl and *Chlorella* ratios were kept the same in all samples. Following that, samples were heated up for 1 hour at temperatures ranging from 20 to 80 °C. To improve gel formation efficiency, different varieties of microalgae were considered to incorporate with *Chlorella*, starch, NaCl, and water in the following trials.

Table 3. Detailed amounts of components in samples.

Sample #	Chlorella (g)	Starch (g)	NaCl (g)	Distilled water (mL)
Sample 1	0.3	0.6	0.6	5
Sample 2	0.3	0.45	0.6	5
Sample 3	0.3	0.35	0.6	5

3.1.3. *Chlorella-k-Carrageenan*-Starch Gel Formation

The microalgae *Carrageenan* was selected to use in combination with *Chlorella* since it generates gel easily with distilled water [23]. To further understand the impacts of starch on *Chlorella* and *k-Carrageenan*, as well as gel formation of *k-Carrageenan*, a sample containing 0.05g *k-Carrageenan*, 0.05g *Chlorella*, 0.3g NaCl, 0.3g starch, and 5mL distilled water was prepared in a 5mL beaker. Amounts were weighted using an analytic balance and all components were transferred into 5mL beakers before adding 5mL distilled water and a magnetic stirrer bar. After heating the sample from 20 to 80 °C around 1 hour at room temperature (20 °C), the sample was transferred to a quartz cuvette to analyze the gel formation features in a more detailed way (Figure 2). In the end, *Chlorella-k-Carrageenan*-Starch sample formed the most gel-like structure in physical terms.

Figure 2. The *Chlorella-k-Carrageenan* (0.05g *k-Carrageenan*, 0.05g *Chlorella*, 0.3g NaCl, 0.3g starch, and 5mL distilled water) sample inside of a quartz cuvette.

3.2. Photometric Analyses

3.2.1. Sol-Gel/Gel-Sol Phases Observation with UV-VIS Spectrophotometer

For this stage of the experiment, all samples from each group were put into quartz cuvettes from Sigma-Aldrich and placed in a HINOTEK 752N Plus UV-VIS Spectrophotometer. For the heating (gel-sol) part, by pouring water into a beaker and heating it on top of the MTOPS MS300HS Hot Plate & Stirrer, a hot water bath was prepared in a 1 L beaker from ISOLAB.

To track the temperature changes, a PeakTech 2010 model digital multimeter was inserted within quartz cuvettes. Finally, one Uni-T brand UTP3315TFL-II model regulated DC power supply and one PHYWE brand Stelltrafo model power supply was utilized to power a smaller 12V motor source, which was used to move water throughout the entire setup was located next to the water bath. The motor source was settled in the voltage range of 4-5V to get constant water circulation and proper data collection.

Water circulation with changing temperatures was achieved by connecting the setup with pipes and a copper coil. The same arrangement was utilized for the cooling (sol-gel) part, by taking out the copper coil and then removing the beaker from the hot plate. Cold water was added in small amounts inside the water bath to speed up cooling and readily cool the circulating water.

4. Results

4.1. Photon Transmission Analyses

The analysis was carried out at 550 and 640 nanometers (nm), with heating and cooling. Absorbance and transmittance (TM) graphs were created using the acquired data. For Sample 1, the following absorbance and transmittance were obtained. Arrhenius plots were also obtained through the transmittance values (Figure 3).

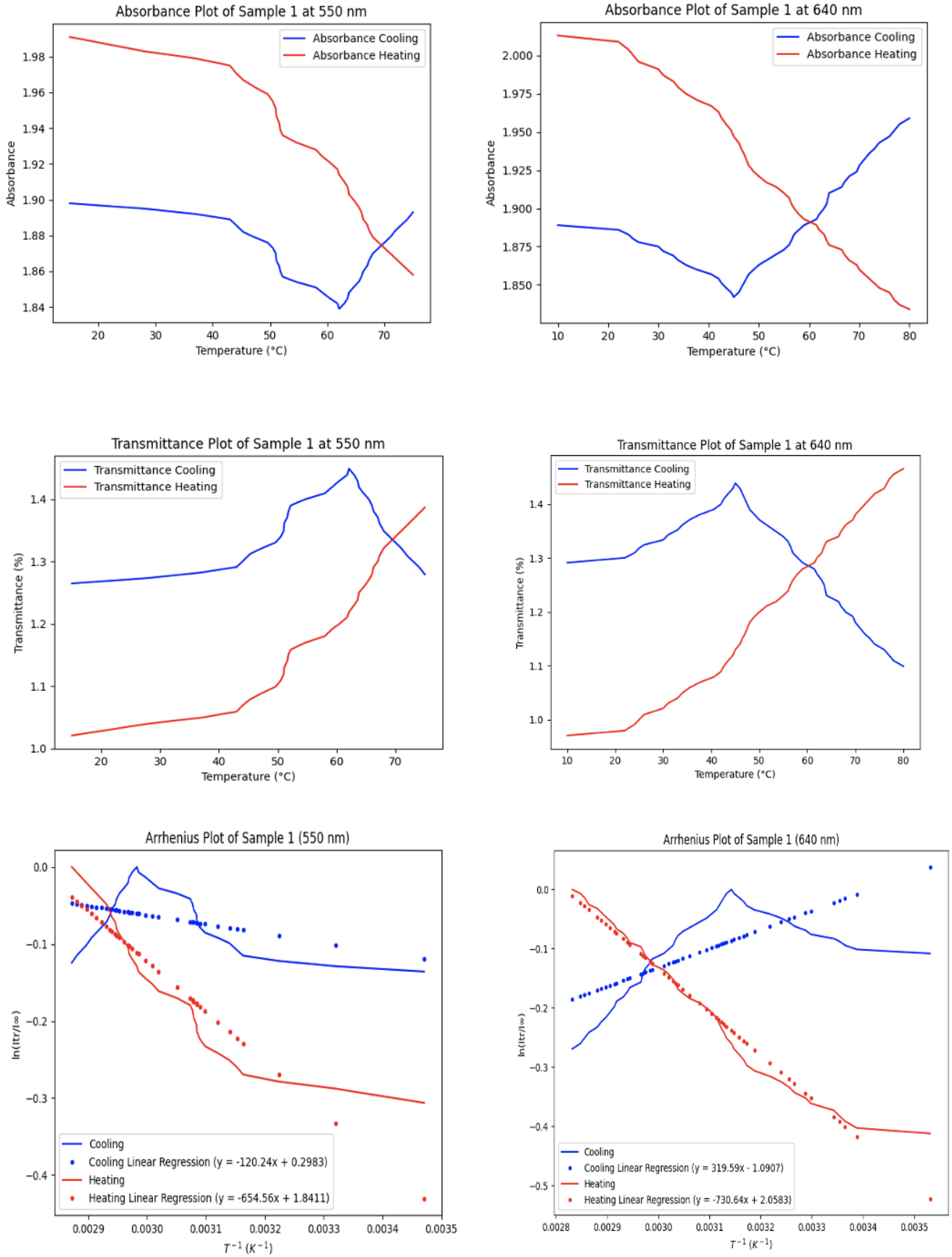
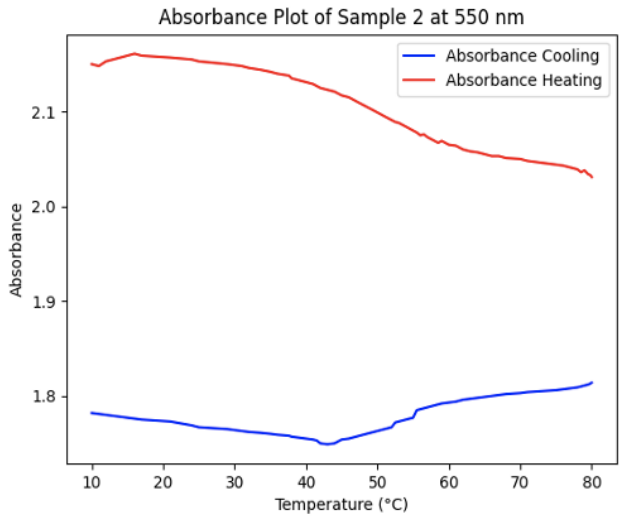
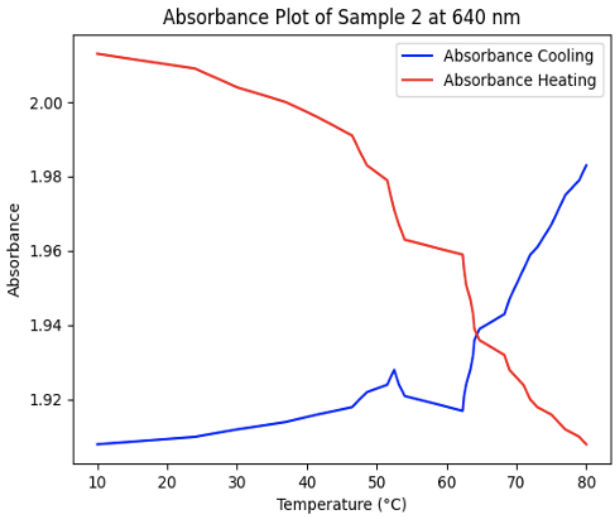
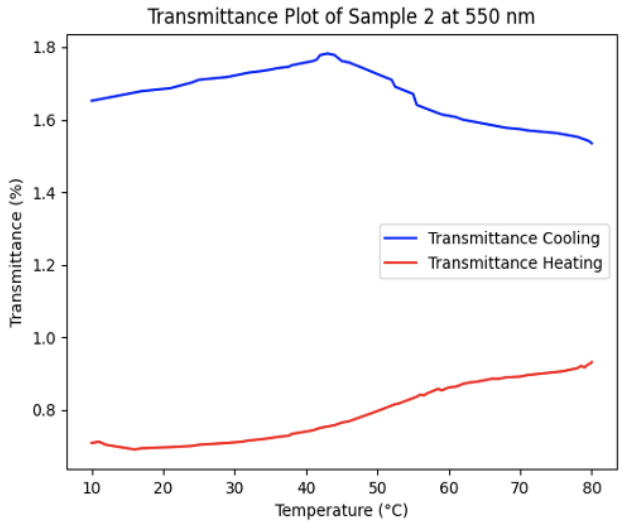


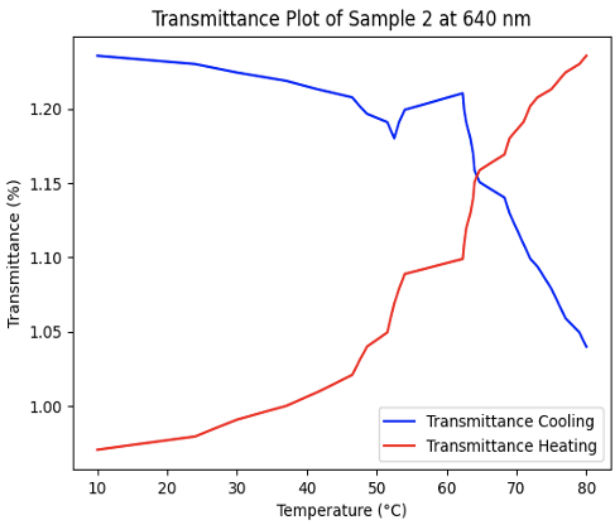
Figure 3. The absorbance, transmittance, and Arrhenius measurements were done under 550 and 640 nm for Sample 1 (0.3g *Chlorella* 0.6g starch 0.6g NaCl, 5 mL distilled water).

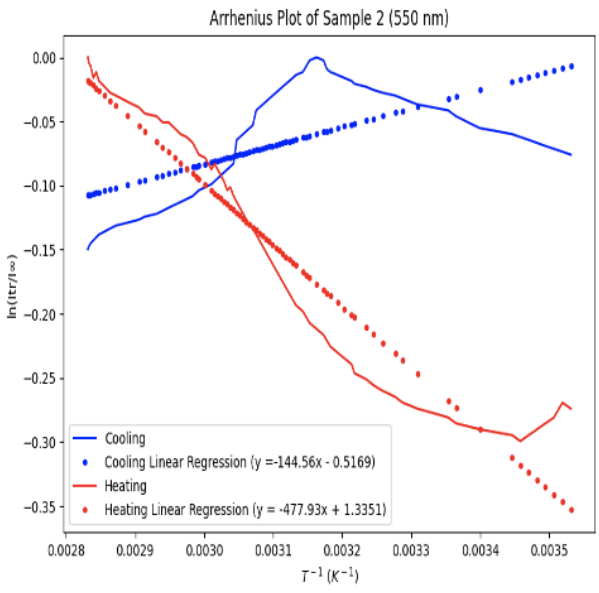
For Sample 2, heating, and cooling under 550 and 640 nm to obtain the absorbance, transmittance, and Arrhenius plots were done (Figure 4).











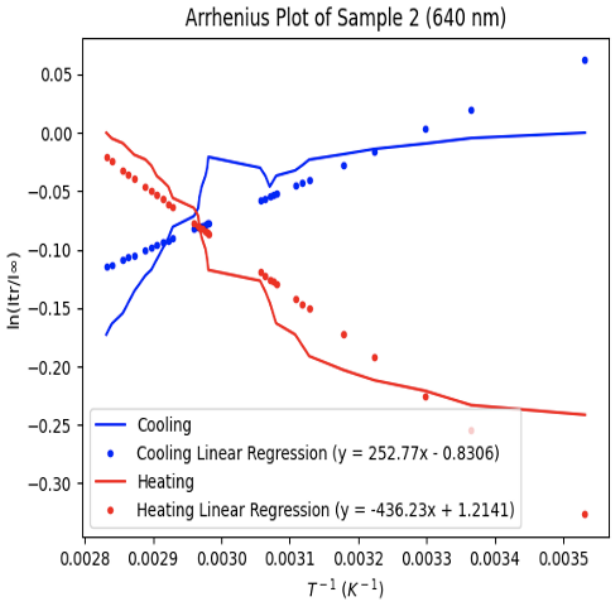
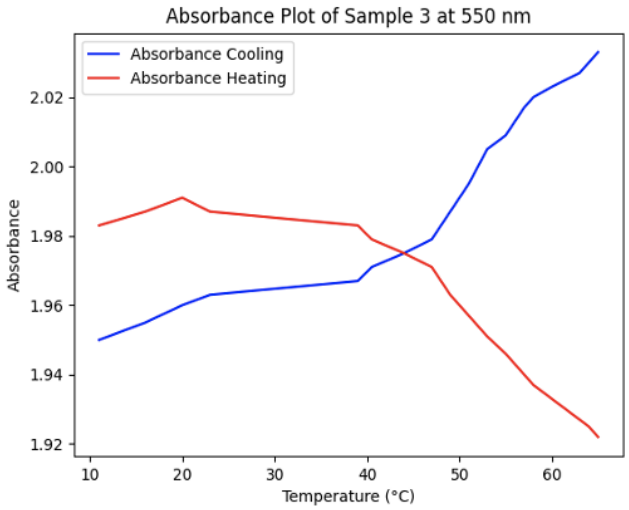
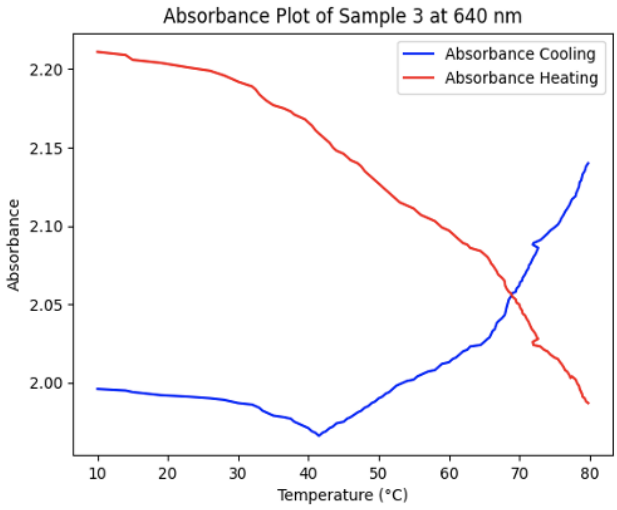
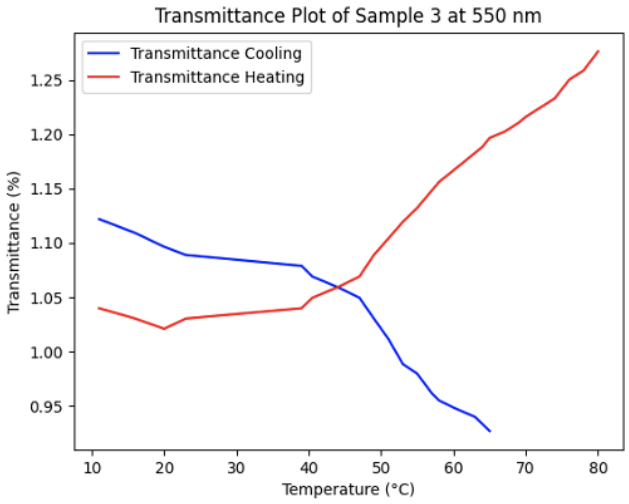


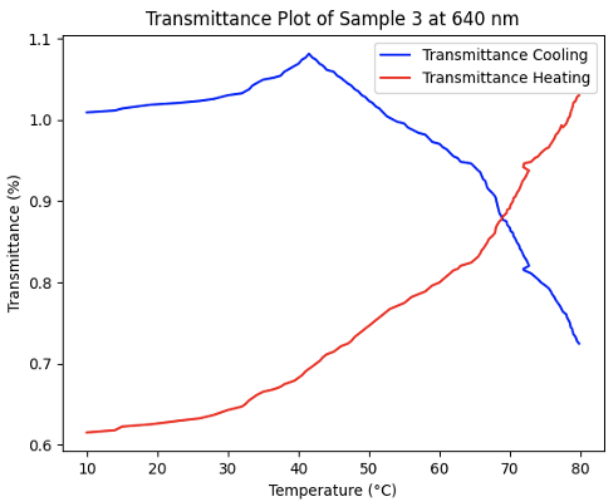
Figure 4. The absorbance, transmittance, and Arrhenius measurements were done under 550 and 640 nm for Sample 2 (0.3g *Chlorella* 0.45g starch 0.6g NaCl, 5 mL distilled water).

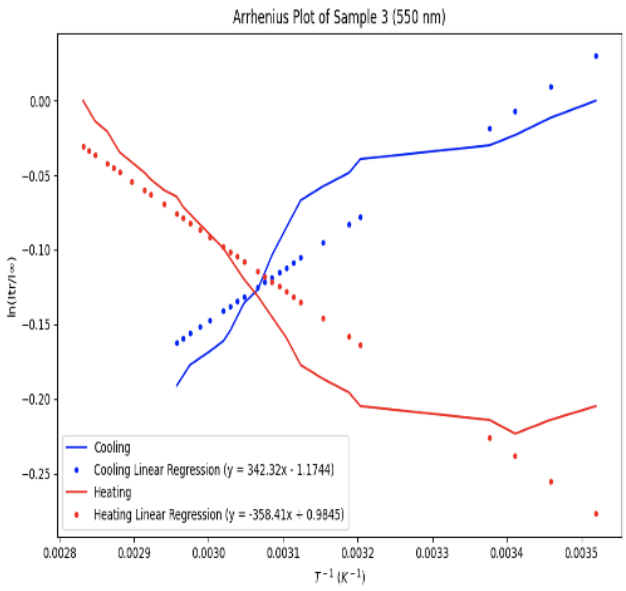
The absorbance, transmittance, and Arrhenius graphs of the heating-cooling for Sample 3 came out as following (Figure 5).











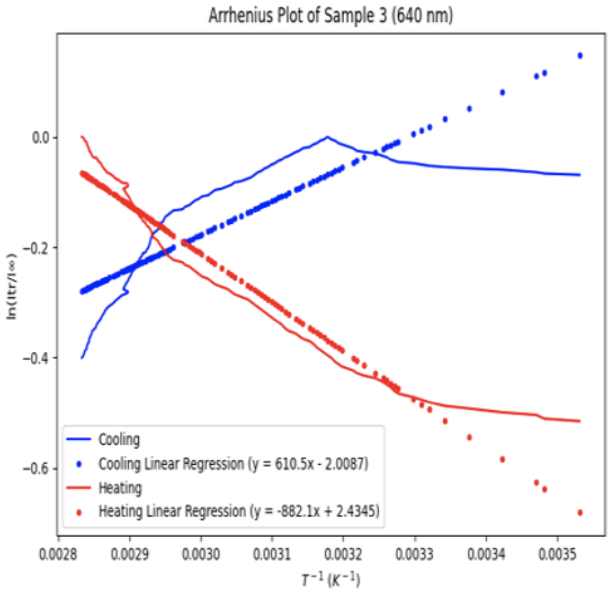


Figure 5. The absorbance, transmittance, and Arrhenius measurements were done under 550 and 640 nm for Sample 3 (0.3g *Chlorella* 0.35g starch 0.6g NaCl, 5 mL distilled water).

When whole photometric analyses for the *Chlorella*-Starch-NaCl group were finished, the absorbance, transmittance, and Arrhenius plots of the sample that contained 0.05g *k-Carrageenan*, 0.05g *Chlorella*, 0.3g NaCl, 0.3g starch, and 5mL distilled water were obtained (Figure 6).

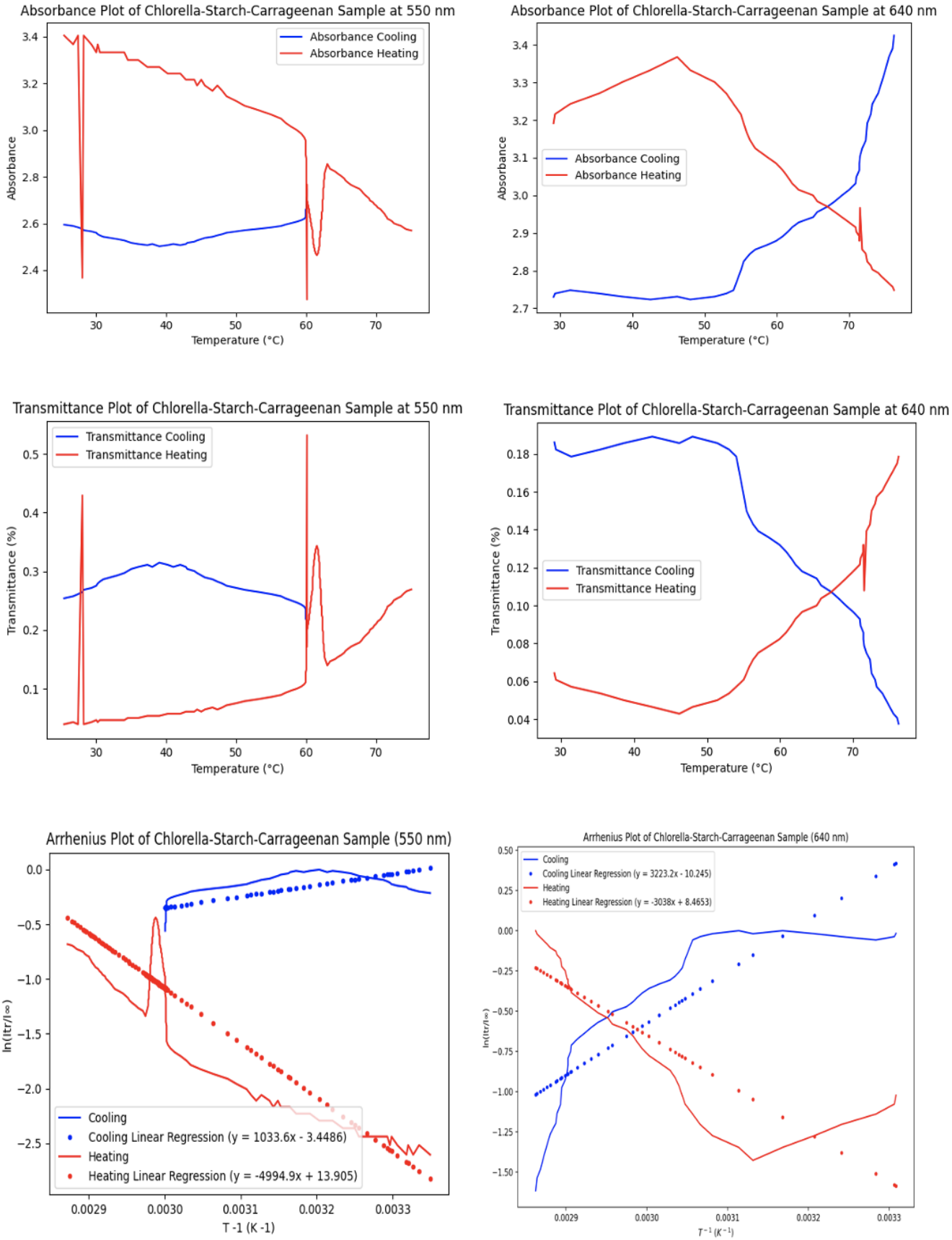


Figure 6. The absorbance, transmittance, and Arrhenius measurements were done under 550 and 640 nm for 0.05g *k-Carrageenan*, 0.05g *Chlorella*, 0.3g NaCl, 0.3g starch, and 5 mL distilled water sample.

Through slope values of Arrhenius plots (Table 4), produced Energy values for Gel-Sol and Sol-Gel transition are calculated in Table 5.

Table 4. Slope values of Arrhenius plots of the samples.

	550 nm		640 nm	
Sample #	**Slope Heating**	**Slope Cooling**	**Slope Heating**	**Slope Cooling**
Sample 1	-654.56	-120.24	-730.64	-319.59
Sample 2	-477.93	-144.56	-426.23	-252.77
Sample 3	-358.41	-342.32	-882.1	-610.5
Chlorella-k-Carrageenan	-4994.9	-1033.6	-3038	-3223.2

Table 5. Gel–Sol (ΔEgs - heating) and Sol–Gel (ΔEsg - cooling) activation energies.

	550 nm		640 nm	
Sample #	**ΔEgs (kJ/mol)**	**ΔEsg (kJ/mol)**	**ΔEgs (kJ/mol)**	**ΔEsg (kJ/mol)**
Sample 1	5.44201184	0.99967536	0.00607454096	2.65707126
Sample 2	3.97351002	1.20187184	3.62681622	2.10152978
Sample 3	2.97982074	2.84604848	7.3337794	5.095697
Chlorella-k-Carrageenan	41.5275986	8.5933504	25.257932	26.7976848

5. Discussion

The initial samples obtained at the beginning of the trials were too liquid. This proved that *Chlorella* and distilled water weren't enough to reach a gel form, which is an important criteria because packaging materials usually have good film forming abilities and gel properties [24]. This could also be caused by the *Chlorella* used in the experiment since it was a commercial product and not pure *Chlorella*. Due to its ability to contribute to gel formation by cross-linking and forming random chains, NaCl was mixed into these samples, however, the outcome was the same [14].

Once starch was added to the samples, significant structural improvements took place. A physical gel-like structure was observed in several samples. The best sample that displayed this was the sample with the *k-Carrageenan.*

As expected, none of the absorbance or the transmittance plots displayed sigmoidal curves. To provide extra proof Arrhenius plots were obtained by the calculation of negative activation energies of the sol-gel/gel-sol processes through the slopes [25]. Overall, only Sample 1 at 640nm and *Chlorella-k-Carrageenan* sample at 640nm showed the expected behavior which was the gel-sol transition having lower activation energy than the sol-gel transition. It is interesting to note that addition of starch decreases the gel-sol activation energy in both wavelengths, on the other hand increases the sol-gel energy during heating and cooling processes respectively. These findings predict that inclusion of starch, accelerates gel-sol transitions however slows down the sol-gel transitions.

To conclude, from the photometric analyses, it could easily be said that the samples showed only physical gel properties.

6. Conclusions

Plastic has been recognized as a danger to the environment for decades, specifically in terms of packaging. Natural packaging alternatives can be considered to reduce their impact. Here, these experiments began with an attempt to obtain a *Chlorella-based* gel to fulfill this purpose. When the gel couldn't be produced through distilled water then *Chlorella*, and materials such as NaCl, *k-Carrageenan,* and starch were added. However, through photometric analyses, it was understood that only the physical gel form was observed. This was caused by the addition of starch. Starch prevented the formation of bonds that would classify the mixture as a chemical gel. In packaging, a non-gel forming material is not favored. The fact that there was no chemical gel formation indicates that starch's addition resulted in a non-favorable packaging material. The resulting mixture can't be used as a packaging material currently. However, since it ended up being extremely dense, it is still a promising step in the research of natural packaging alternatives.

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