Comparing Vitamin C and Vitamin E in Improving the Photostability of a Retinol Serum Exposed to Heat Degradation

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Retinol, a form of Vitamin A, is a popular ingredient in skincare products for its anti-aging effects. However, in most cosmetic formulations, retinol destabilizes quickly, due to exposure to heat, light, oxygen and pH. To combat retinol degradation, many preservatives have been examined, including antioxidants like Vitamin C and E. Still, studies investigating the supplementation of Vitamin C and E in retinol formulations have mostly focused on stabilizing retinol against UV light alone and have yet to explore the effects of adding these vitamins on other stressors, such as heat. The purpose of this study was to compare the abilities of Vitamin C, Vitamin E, and a combination of both on improving a retinol serum's photostability when exposed to heat. It was hypothesized that adding both Vitamin C and E to the retinol serum before heat degradation would most effectively improve the retinol's photostability, because both vitamins are lipid-soluble, exhibit antioxidative properties, and work synergistically to improve stability. Two control groups were made: retinol with no heat or vitamins, and retinol with heat and no vitamins. Three experimental groups were made, all of which were heat degraded: retinol and Vitamin C, retinol and Vitamin E, and retinol with both vitamins. Four of the groups were placed in a drying oven for 20 days at ~65 ℃. The UVA intensity (mW/m^2) of the UVA light of each mixture was measured using a UVA Sensor. The results of a one-way ANOVA test ($\alpha = 0.05$) suggest there were significant differences between the control groups and each of the experimental groups, (ANOVA[$F(4,295) = 30.709$, $p < 0.001$]). A post-hoc Tukey test found significant differences between the two control groups, the first control and all experimental groups, the second control and all experimental, and between each experimental group except for Vitamin E vs. Vitamin C and E.

Introduction

Vitamin A and its derivatives, also known as retinoids, are fat-soluble compounds used in topical formulations, but also found naturally in the human body. Many retinoids, such as retinol and retinyl esters, are synthesized in human intestines and are abundantly found in the liver. Inside the body, retinol can moderate gene expression and cell differentiation, and is also essential for vision[1,2,3]. However, from a cosmetic perspective, retinol is an extremely effective and potent skincare ingredient used in many commercial products. It is known to increase collagen production and cell proliferation in the epidermis of the skin. In addition, retinol can also prevent acne, unclog pores, and control oil production[4]. However, one of retinol's most prominent benefits is its ability to reduce the appearance of wrinkles and fine lines caused by UV light from the sun. Human studies have shown that retinol creams can significantly improve skin texture, pore size, and erythema, or sunburn[5]. Another clinical study concluded that participants whose skin had been treated with retinyl retinoate saw a decrease in periorbital wrinkles, or wrinkles around the eyes, due to higher collagen synthesis in the skin[6].

Although retinol has countless benefits, many factors impacting its stability severely limit its effectiveness. Retinol is extremely sensitive to UV light, heat, oxygen, and even pH when it is not stabilized properly, meaning that it degrades quickly and becomes ineffective, which can sometimes even be harmful to skin[7]. When exposed to air and UV light, retinol will either oxidize or decompose rapidly, altering its chemical makeup and gradually increasing its phototoxicity[3]. Similar to air and sunlight, heat can significantly change the composition of retinol, making it less biologically active[8]. For example, a degradation kinetics study found a 40-100% decline in retinol stability at 40 °C after 6 months[9]. In order to reap the immense benefits that retinol possesses, various methods of retinol stabilization have been explored, such as the addition of lipid-soluble antioxidants, like Vitamin C and E, that can improve the overall stability of cosmetic retinol formulations.

Vitamin C, also known as ascorbic acid, is a well-studied and popular antioxidant used in skin care products. It can reduce inflammation, even out fine lines and wrinkles, and protect the skin from UV damage[10]. Topical application of a 5% Vitamin C cream on the sun-exposed upper chest and forearm of 19 females found significant improvement in skin structure and collagen production, as well as a decrease in deep skin furrows caused by the sun exposure[11]. Aside from its benefits on the skin, many chemically modified forms of Vitamin C have been formulated to increase antioxidant activity and stability in other topical formulations, namely retinol[12]. One modified derivative of Vitamin C called tetrahexyldecyl ascorbate (THDA) has long fatty acid chains that give it enhanced lipophilicity, making it compatible with retinol, which is fat soluble[12]. In a clinical trial using 0.5% topical retinol and 30% THDA moisturizer, patients saw statistically significant reductions in wrinkles, hyperpigmentation, and brightness of the skin[13], suggesting that retinol in combination with Vitamin C improves the effectiveness of the cream, meaning the retinol was successfully stabilized and able to perform.

Vitamin E is another powerful antioxidant implemented into skincare formulations. There are countless classes of molecules referred to as "Vitamin E", but the most abundant type, α-tocopherol, is found in nuts and oils, and is also naturally synthesized in the body[14]. Tocopherol is fat-soluble and exhibits photoprotective properties, meaning it can prevent UV-induced free radical damage in the skin. In addition, because tocopherol is lipophilic, it has increased molecular stability when exposed to heat, light and air[15], which is why retinol is often supplemented with it. A 0.1% retinol and 0.1% Vitamin E gel reduced dark under-eye circles substantially[16], once again indicating that Vitamin E is highly effective in combination with retinol, possibly more than Vitamin C.

By supplementing retinol with these antioxidants, the retinol can remain chemically stable against UV light for longer amounts of time[17]. In addition, the usage of lipid-soluble compounds has been shown to increase retinol photostability as well as thermal stability. A retinol emulsion with a 10% soybean oil concentration demonstrated high levels of UV stability due to the fact the lipid droplets scatter the light[18]. In addition, solid lipid nanocarriers, which encapsulate retinol in a lipid bilayer, were found to degrade slower than retinol in a methanol solution[17], emphasizing the importance of lipids in stabilizing retinol from both light and heat. Because both lipids and antioxidants (Vitamin C and E) are effective in stabilizing retinol, supplementing retinol with oil-soluble forms of Vitamin C and E (THDA and tocopherol) could potentially improve retinol's stability most optimally.

In order to accurately quantify and measure the stability of retinol formulations, many studies utilize an irradiation technique using UVA light to test the absorbance of the retinol. There are many methods of testing UV absorbance, such as using a UV light sensor, which can measure the

intensity of UV light at a certain wavelength range. Most irradiation equipment measure UV intensity, or strength of the UV light, in milliwatts (mW) or millijoules (mJ) per centimeter squared (cm^2) of surface area that is being irradiated[19]. Higher light intensity suggests a loss of photoprotectivity in the retinol serum over time, meaning that the strength of the UV light passing through the retinol also becomes greater, and it degrades[20]. Conversely, a decrease in UV intensity would most likely indicate improved stability and absorbance ability of the retinol, because the strength of the UV light would be minimized, possibly demonstrating the abilities of the vitamins to protect the retinol from degradation.

Existing literature about supplementing retinol with fat-soluble forms of Vitamin C and Vitamin E heavily focuses on its stability against UV light or on its anti-aging effects on human skin. However, less is known about the effects of Vitamin C and Vitamin E on the thermal stability of a retinol serum through the UV irradiation process as a way of measuring stability. Although shelf-life tests have been conducted on retinol gel-cream formulations with added Vitamin E and C[21], its chemical stability was determined through high performance liquid chromatography (HPLC), and not UV irradiation. The present study aims to directly evaluate the abilities of Vitamin C and Vitamin E in improving the stability of a retinol serum from heat degradation using a UV irradiation technique to measure light intensity.

Literature Review

UVA Absorbance of Retinol

Although retinol degrades when exposed to UV light, this occurs only after long durations of time. In fact, for short periods of time, retinol can absorb UV light at a peak wavelength of 325 nanometers (nm), which falls under the category of UVA light[22], therefore being the wavelength range used in the present study. Tolleson et al. (2005) found that a retinol solution exposed to 5 minutes of UVA light at wavelength 320 nm generated significantly less free radicals compared to the retinol solution irradiated for 30 minutes[3]. In a 2003 study by Antille et al., topical retinyl palmitate was applied to the back of hairless mice, which were then exposed to 2 hours of UV light. It was concluded that after 2 hours of exposure, the topical retinyl palmitate significantly inhibited the formation of thymine dimers, or UV photolesions, in the mice epidermis[23]. These findings demonstrate that because retinol has short-lived UVA absorbance properties, UVA light can be used in the irradiation process of testing retinol's stability if done within a short time frame.

Thermal Stability Testing of Retinol (Heat Degradation)

Testing retinol's thermal stability and shelf life through heat degradation is a common procedure in cosmetic testing, but studies vary in the temperature and time at which the retinol is degraded. In a study testing the thermal stability of a novel hybrid retinol derivative, bis-retinamido methylpentane, solutions were stored at 4 ℃, 25 ℃, and 40 ℃ respectively. The bis-retinamido methylpentane solution degraded more than 50% after 120 h at 25 ℃[24]. Chaudhuri (2015) noted that most commercial retinol products start to rapidly degrade at 40 ℃, so the study incubated retinol mixtures at 37 ℃ overnight to accomplish heat degradation[25]. Another study investigating the thermal stability of retinol-loaded lipid nanoparticles stored the mixtures at 25 °C and 40 °C, while the control group was stored at 4 ℃ for comparison of degradation[26]. Stability tests were performed by Wang et al. (2023) in order to determine the effectiveness of novel, environmentally friendly, antioxidants to stabilize retinol. The retinol formulations were stored in opaque jars for two months at 4 ℃ and 45 ℃, where the 45 ℃ formulation with no antioxidants lost 31% of its retinol content[27]. Park et al. (2019) determined the effect of adding the antioxidant EDTA to retinol nanoemulsions on thermal stability by storing emulsions at 4 ℃, 25 °C, and 40 °C for one week[18]. After examining these studies, it is evident that storage temperature and length of heat degradation varies vastly for thermal stability tests. However, each method of heat degradation catered to the specific needs of the experiments. For this reason, the present study degraded the retinol mixtures at a significantly higher temperature but stored it for only 20 days before collecting data.

Synergistic Effects of Retinol and Vitamins C and E as Fat-Soluble Antioxidants

Despite the fact that many studies use UV irradiation to test retinol stability for short periods of time, for practical cosmetic use, retinol must be supplemented with antioxidants to protect its stability for long term usage. According to previous studies, because retinol is lipid soluble, supplementing it with other lipid-soluble substances can significantly increase its thermal stability and stability against UV light[18]. Furthermore, prior literature concluded that using Vitamin C and E, which are antioxidants, reduced heat and UV light induced degradation up to 90%, according to a HPLC analysis[28]. Similarly, another study tested the chemical and photostability of a 1% retinyl palmitate formulation with Vitamin C and E to determine the two vitamins' synergistic benefits. The study concluded that the formulation with a combination of both vitamins had a lower degradation rate compared to the formulations containing only one vitamin[21]. In a study by Herndon et al. (2016), a 0.5% retinol and 30% vitamin C cream with Vitamin E was tested on 44 participants in a dual product regimen. The results of the study suggested that participants' photodamage and hyperpigmentation substantially decreased after only 4 weeks of the treatment[13]. Gaspar et al. (2007) tested a 0.6% retinol, 2% Vitamin E, and 2% Vitamin C formulation with photostable UV filters, and found that it enhanced Vitamin A photostability after being irradiated with UVA/UVB light[29]. These previous studies reveal a gap in the research, indicating that most previous studies examine Vitamin C and E's antioxidative properties as being able to prevent UV light degradation, but rarely address their effects on solely thermal degradation.

The purpose of the present study was to explore the abilities of Vitamin C (THDA), Vitamin E (tocopherol), and a combination of both in improving the photostability of a cosmetic retinol serum after direct heat degradation. It was hypothesized that if a retinol serum is degraded by high heat, then adding Vitamin E and Vitamin C to it before heat degradation would result in the lowest UVA intensity values of the retinol serum, due to the fact that both vitamins are oil-based compounds with antioxidative properties, and work synergistically to improve stability. Three separate experimental groups were prepared by dividing the retinol serum into separate amber bottles. The first control group consisted of retinol with no vitamins and no heat degradation, and the second control included retinol with no vitamins but was degraded with heat. Four of the groups were placed in a drying oven for around 20 days. The photostability of all five retinol groups were quantified using a UVA Sensor and UVA lamp, which produced UVA intensity values in milliwatts per meter squared (mW/m^2).

Methods

Before starting experimentation, the retinol serum, THDA, and Vitamin E were stored in a cool, dark environment to preserve the products. All experimentation occurred in areas with little direct sunlight in order to protect all products from breaking down. The irradiation part of the method was conducted in a dark room with no outside light except for the light from the UVA lamp to ensure that the UV intensity readings were coming from the UVA light alone, which was adapted from the methods of Bonda and Zhang (2013). All 15 amber bottles were labeled according to the five groups ahead of time to make experimentation more efficient. The five groups had three bottles of retinol mixture each.

Using a 100-1000 µl micropipette and tips, 5000 µl of the retinol serum was pipetted into each of three amber bottles. To minimize the retinol's exposure to oxygen, each group of bottles were prepared one at a time and sealed immediately after contents were pipetted. For one control group, only the retinol serum was added to the bottles, and then was immediately placed in a cardboard box away from light for around 20 days. For the group with THDA, 5000 µl of the retinol serum was pipetted into each of the three bottles. Then, using a 20-200 µl micropipette and tips, 250 µl of the THDA (5% concentration) was pipetted into each bottle and immediately sealed. The bottles were vigorously shaken by hand to distribute the THDA throughout the serum. This process was repeated for the Vitamin E group, with 250 µl of Vitamin E in 5000 µl of the retinol serum per amber bottle. For the group with THDA and Vitamin E, 125 µl of THDA and 125 µl of Vitamin E Oil were micropipetted into each bottle with the 20-200 µl micropipette and tips. These three bottles were also vigorously shaken after sealing.

Beforehand, the drying oven was set to ~65 ℃, or around 150 °F. According to Wang et al. (2023) and Temova Rakuša et al. (2020), retinol typically degrades at temperatures between 40-45 ℃[9,27]. However, because these studies degraded retinol over several months and the present study degraded retinol over approximately 20 days due to time constraints, a higher temperature was used to produce the same degradation effect. A thermometer was kept inside the drying oven at all times to ensure a constant and accurate temperature. After all groups of bottles were prepared, four of the groups, including the second control group with just retinol serum, were placed into a drying oven for approximately 20 days. After 20 days, the four groups of bottles were removed from the drying oven and allowed to cool to room temperature.

To begin the irradiation procedure, 15 glass microscope slides were labeled according to the appropriate groups. Using a ruler and a Sharpie, a 2 cm by 2 cm square was drawn onto each slide. Using the 100-1000 µl micropipette and tips, 500 µl of retinol mixture from each bottle was pipetted onto each of the 15 slides. The glass pipette tips from the amber bottles were used to distribute the mixture evenly across the glass slide. This method of spreading the retinol product on glass plates was adapted from Ahmad et al. (2011), who spread retinol cream on rectangular glass plates before irradiating them in a dark chamber to test retinol stability[30]. Once this was completed, the UVA lamp setup was prepared. The 75 W UVA light bulb was screwed into a lamp fixture that had a clamp apparatus attached to it. This lamp fixture was then clamped onto a ring stand. The Vernier UVA Sensor was fixed around 10 cm underneath the lamp fixture using a utility clamp attached to the ring stand[30]. The UVA Sensor was plugged into the LabQuest2 program and turned on. This entire setup is depicted in Figure 2. The LabQuest2 was set to collect a UV intensity reading (mW/m^2) every 5 seconds for 100 seconds, producing 20 trials per microscope slide. Each of the five retinol groups had three microscope slides, so a total of 60 trials were conducted per group. The UVA intensity readings for each group were recorded in a spreadsheet for later data analysis.

A one-way ANOVA inferential test was conducted on the data to determine if the data was statistically significant. This particular inferential test was used because there was only one independent variable in the experiment (addition of vitamins), and would accurately display whether there was a statistical difference between the different vitamin combinations and UVA intensity. The experimental design diagram is displayed in Figure 1.

Results

The raw data displaying all the UVA intensities of the two control groups and three experimental groups is presented in Appendix A. The raw data were used and interpreted to calculate the means (*M*), ranges, and standard deviations (*SD*) for all five groups. Table 1 includes the mean, range, and standard deviation for all five retinol groups, including two control groups and three experimental groups. The retinol serum with both Vitamin C and Vitamin E added to it before heat degradation produced the lowest mean UVA intensity ($M = 64.283$), range and standard deviation ($SD = 4.267$). The second control group, the retinol serum with no vitamins before heat degradation, produced the highest mean ($M = 87.617$), range, and standard deviation ($SD = 18.487$). Other than the control groups, the retinol serum with Vitamin C added before heat degradation had the highest mean ($M = 76.783$) and standard deviation ($SD = 12.367$) out of the three experimental groups.

Figure 3 depicts the minimum, first quartile, median, third quartile, and maximum UVA intensity readings in mW/m^2 for each of the five retinol groups in a box plot. The boxplot chart shows the minimum, first quartile, median, third quartile, and maximum of the data in each group. As shown in the chart, between the two control groups, the retinol group that was degraded with heat and had no vitamins added resulted in the highest median, third quartile, and maximum UVA intensity readings out of all five groups. Comparing the control groups, the heat degraded retinol with Vitamin C had the highest median, third quartile, and maximum, and the heat degraded retinol with Vitamin C and E had the lowest median, third quartile and maximum UVA intensity readings.

Figure 4 displays a line graph of one 100 second interval of the raw UVA intensity values. One data point was collected every 5 seconds for 100 seconds per glass slide of retinol (3 per group). In other words, the line graph displays 20 trials of the raw data, or one slide's worth per retinol group. The data was presented in this way due to the separation of time between the 60 trials. In Figure 4, it is evident that the line showing the second control group (heat, no vitamins), has the highest peak UVA intensity compared to the rest of the groups, and continues to maintain higher values over the 100 second period. The line depicting the retinol with Vitamin C and E shows the lowest UVA intensity values, with the values remaining relatively constant.

Table 2 depicts the one-way ANOVA test conducted to determine whether there was statistical significance between the mean UVA intensities of the five retinol groups. An alpha value of 0.05 was used. The null hypothesis (H_0) stated that the mean UVA intensities of all five groups were equal, while the alternative hypothesis (H_a,) stated that at least one out of five mean UVA intensities would not be equal. There was a statistically significant difference between at least one of the mean UVA intensities as determined by one-way ANOVA ($F(4,295) = 30.709$, $p < 0.001$), at □ = 0.05. This suggests that the addition of vitamins resulted in lower mean UVA intensity readings. The decision to reject the null hypothesis was made, and a post hoc Tukey test was conducted in order to determine between which groups the differences exist.

Table 3 depicts the mean differences in UVA intensity between each of the five retinol groups. Each of the mean differences were compared to the calculated Tukey value, or critical value, to determine statistical significance at = 0.05. Statistical significance was determined if the mean difference between the groups was greater than the critical value. The results of the post hoc Tukey Test show that there was statistical significance between the two control groups, due to the mean difference of 8.150 being greater than the Tukey value of 6.460. Similarly, there was statistical significance between the second control group (heat, no vitamins) and all the experimental groups. Between the control groups themselves, there was statistical significance between the Vitamin C and Vitamin E mixtures', and also between the Vitamin C mixture and then mixture with both Vitamin C and E. There was no statistical significance between the Vitamin E group and the Vitamin C and E group, as well as between the first control group and the Vitamin C group.

Discussion

The purpose of this study was to determine how Vitamin C, Vitamin E, and a combination of both vitamins compared in improving the photostability of a retinol serum exposed to heat degradation. It was hypothesized that if a retinol serum is degraded by high heat, then adding Vitamin E and Vitamin C to it before heat degradation would result in the lowest UVA intensity values of the retinol serum, due to the fact that both vitamins are lipid-soluble compounds with antioxidative properties, and work synergistically to improve chemical stability. The results of this study indicate that the addition of both Vitamin C and E to a heat degraded retinol most optimally improved its photostability compared to the other retinol groups. Therefore, the hypothesis was supported. Furthermore, the results of the one-way ANOVA test indicated that there was statistical difference between the mean UVA intensities of the five retinol groups, with $p < 0.001$ at $= 0.05$.

According to Table 1, out of all five retinol groups, the second control (heat, no vitamins), had the highest mean, range, and standard deviation. When comparing the second control group to the first (no heat, no vitamins), the first control group had a significantly lower mean UVA intensity, indicating that the presence of high heat and no protective vitamins in the second control group effectively degraded the retinol, allowing more UVA light to be transmitted through the retinol. Although all three experimental groups had lower UVA intensities compared to both control groups, there was variation between the three groups. The Vitamin C and retinol had the highest mean, range, and standard deviation out of the three experimental groups, indicating that it was least effective in improving the retinol's photostability compared to the Vitamin E and Vitamin C and E groups. The Vitamin C and E had the lowest mean UVA intensity out of all groups and between the three experimental groups, suggesting that the combination of Vitamin E and Vitamin C in a retinol serum most effectively lowered the strength of UVA light passing through the serum, illustrating the synergistic effects of both vitamins on retinol's photostability. This indicates that the vitamins exhibited antioxidative and photoprotective properties against the UVA light, which meant that it stabilized the retinol serum to the extent that it was able to absorb UVA light most effectively.

In Figure 3, it is evident from the boxplot that the heat degraded retinol with no vitamins (red) had the highest maximum UVA intensity value, and all three experimental groups (blue, orange, purple) had significantly lower minimum, first quartile, median, third quartile, and maximum UVA intensity values compared to the control groups. This once again demonstrates that the addition of Vitamin C, Vitamin E, or a combination of both, did produce lower UVA intensities compared to the two retinol groups that either had no heat degradation or vitamins, or heat degradation and no vitamins. More specifically, the Vitamin C and E boxplot is positioned lowest on the graph, meaning it had the lowest minimum, first quartile, median, third quartile, and maximum values, and therefore most effectively absorbed the UVA light and improved photostability of the retinol serum with its antioxidative properties.

This trend of decreasing UVA intensities with the addition of vitamins, or, more specifically, a combination of both, is also seen in Figure 4. Figure 4 depicts a line graph of continuous UVA intensity readings (mW/m^2) over the course of 100 seconds. The Vitamin C and E group produced consistently lower readings compared to all other groups, as seen by the orange line which falls below the other four lines. Due to Vitamin C and E's lipophilicity and photoprotectivity, the combination of the vitamins in the retinol serum was able to protect the retinol against thermal degradation, resulting in its ability to absorb more UVA light than the standard retinol serum. The one-way ANOVA test conducted in Table 2 suggests that there was sufficient evidence to reject the null hypothesis, meaning that there was statistical significance between at least one of the mean UVA intensities, ($F(4,295) = 30.709$, $p < 0.001$). In other words, the one-way ANOVA test indicates that the differences between the mean UVA intensities of the five retinol groups are statistically significant, meaning not likely due to random chance.

However, a post hoc Tukey test was conducted to further investigate where the statistically significant differences in mean UVA intensity existed between the groups (Table 3). There was statistical significance between the two control groups, implying that the difference in their mean UVA intensity values showed effective heat degradation of the retinol serum, which was crucial in accurately comparing the control groups to the experimental groups. In addition, all experimental groups except for Vitamin C showed statistically significant differences between their means and the mean of the first control group (no heat or vitamins). This result is supported by research done by Swindell et al. (2021), who found that Vitamin C, or THDA, can prevent lipid degradation, but is very sensitive to degradation by oxygen[31]. In the present study, the Vitamin C group may not have been as effective due to the THDA being briefly exposed to oxygen prior to the heat degradation of the retinol mixture. When comparing the heat degraded retinol with the two control groups, all three groups showed statistical significance between the mean UVA intensities. This demonstrates that all additions of the vitamins improved the photostability of the retinol serum exposed to high heat, but at differing levels of effectiveness. All three experimental groups' mean differences were greater than the Tukey value of 6.46, but the Vitamin C and E group showed the greatest mean difference with the second control group, with a value of 23.334, suggesting that the Vitamin C and E group performed most optimally in improving photostability after heat degradation.

The synergistic effects of Vitamin C and Vitamin E illustrated by the present study is supported by previous literature. A stability study done by Rozman and Gašperlin (2007) found microemulsions containing lipophilic vitamin E and hydrophilic vitamin C to be highly chemically stable, due to microemulsion structure influencing both vitamins to stabilize each other under UVA radiation and oxidative stress[32]. This notion is supported by Burke (2007), who found that combining the two vitamins in a cosmeceutical formulation is synergistic because vitamin C regenerates oxidized vitamin E, which serves to protect the skin from UV damage[33].

The present study was subject to several limitations, including limited time and the lack of a professional laboratory setting. Firstly, as executed by many retinol stabilization studies, such as Wang et al.'s (2023), the retinol formulation's stability was tested for 2 months at 45 °C[27]. However, due to time constraints on the present study, the retinol mixtures were degraded for only around three weeks. Secondly, due to the absence of professional laboratory equipment, the retinol serum and vitamins had to be briefly exposed to oxygen and small amounts of artificial light before being placed into the amber bottles, which could have led to slight degradation of the vitamins, especially THDA, before heat degradation.

Throughout experimentation, several potential sources of error could have altered the study's results. When micropipetting out the retinol serum from their bottles into amber bottles, there were several instances where the serum did not completely fill the micropipette tip due to the high viscosity of the serum. This issue also occurred with the micropipetting of the Vitamin E. Furthermore, when the retinol mixtures were micropipetted out of the amber bottles onto the microscope slides, some of the mixture did not transfer to the slide, and was instead stuck inside the tip. These inconsistencies in micropipetting could have slightly altered the amount of mixture on each slide, and could have resulted in slightly inaccurate UVA intensity readings.

To reduce the likelihood of possible errors, there are several procedural improvements that could be made. The retinol mixtures could have been placed in the drying oven for a longer period of time, such as a one to two months, at a lower temperature for a more accurate and realistic heat degradation process, similar to Wang et al. (2023), as previously mentioned. Additionally, the viscous retinol serum and Vitamin E oil could have been slightly warmed or diluted with neutral liquids to thin out the formulations and produce more precise pipette measurements. An

equivalent approach was utilized by Papageorgiou et al. (2007), who diluted their retinol formulations in absolute ethanol before irradiating them[34].

The results of the present study imply that incorporating Vitamin C and Vitamin E into retinol formulations has the potential to effectively improve the photostability of the formulation against common degradation factors like heat. Using these popular and affordable vitamins to enhance the quality and stability of retinol serums can increase the shelf life of commercial retinol products, making them more long-lasting and beneficial to consumers. Furthermore, the antioxidant activity and photoprotective properties exhibited by both vitamins can serve to protect the skin from UV damage while still reaping the myriad of benefits that retinol has to offer. Overall, the extensive and beneficial implications of this study calls for deeper investigation into commercial retinol products and their thermal stability, which is part of the gap in literature that this study aimed to fill.

To further explore methods of improving retinol stability and shelf life and substantiate the results of the present study, additional research can be conducted. Model organisms like mice and even human subjects can be used to gain a better understanding of the way Vitamin C and E and retinol interact with the skin, and how they respond to environmental stressors like heat and UV exposure. Another option is to delve deeper into improving the chemical stability of retinol serums by exploring the encapsulation of retinol in lipid nanocarriers, which is a novel approach to protecting retinol from degradation.

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Figures and Tables

Figure 1. Experimental Design Diagram

Title of the Experiment

Comparing Vitamin E and Vitamin C in Improving the Photostability of a Retinol Serum Exposed to Heat Degradation

Hypothesis

If a retinol serum is degraded by high heat, then adding Vitamin E and Vitamin C to it before heat degradation would result in the lowest UVA intensity values, due to the fact that both vitamins are oil-based compounds with antioxidative properties, and work synergistically.

Independent Variable

Addition of vitamins before heat degradation

Levels of Independent Variable	**Retinol serum under normal conditions**	**Neither vitamin added before heat**	**Addition of Vitamin E before heat**	**Addition of Vitamin C before heat**	**Addition of Vitamin C and E before heat**
Number of Repeated Trials	60	60	60	60	60

Dependent Variable

UVA Intensity (mW/m^2)

Constants

Amount of retinol, time under UVA light, brand of retinol used, concentration of vitamins, temperature and time in drying oven

Control Group

Retinol serum in normal conditions (no heat)

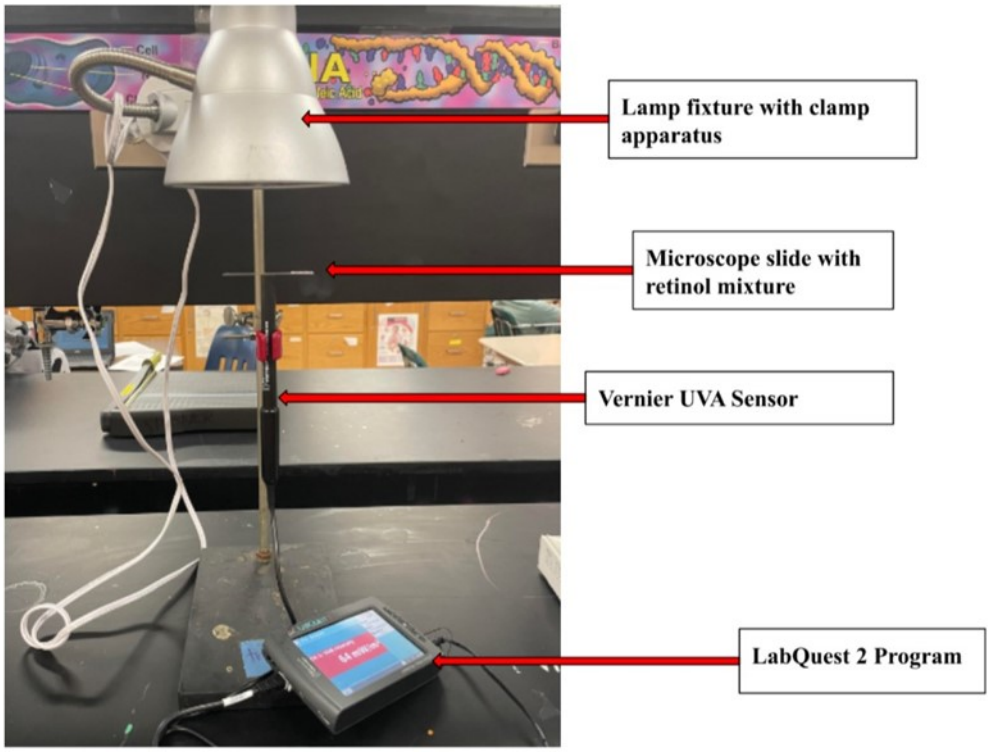

Figure 2. Lamp Setup for UVA Intensity Measurements. *Note.* This figure depicts the UVA irradiation setup and its individual parts, including the lamp fixture, which held the UVA light bulb, the microscope slide with the retinol and vitamin mixtures, the Vernier UVA Sensor, and the Labquest2 Program, which measured and recorded the UVA intensity.

Table 1. Mean, Range, and Standard Deviation of UVA Intensity Values in mW/m^2

Statistic	No heat or vitamins (Control)	No vitamins (Control)	Vitamin C (Test)	Vitamin E (Test)	Vitamin C and E (Test)
M	79.467	87.617	76.783	68.383	64.283
Range	49	54	39	39	14
SD	15.257	18.487	12.367	9.265	4.267

Note. This table shows the combined mean, range, and standard deviation of each of the five retinol groups over three separate 100 second time periods, with each 100 second time period being split into 5 second UVA intensity readings.

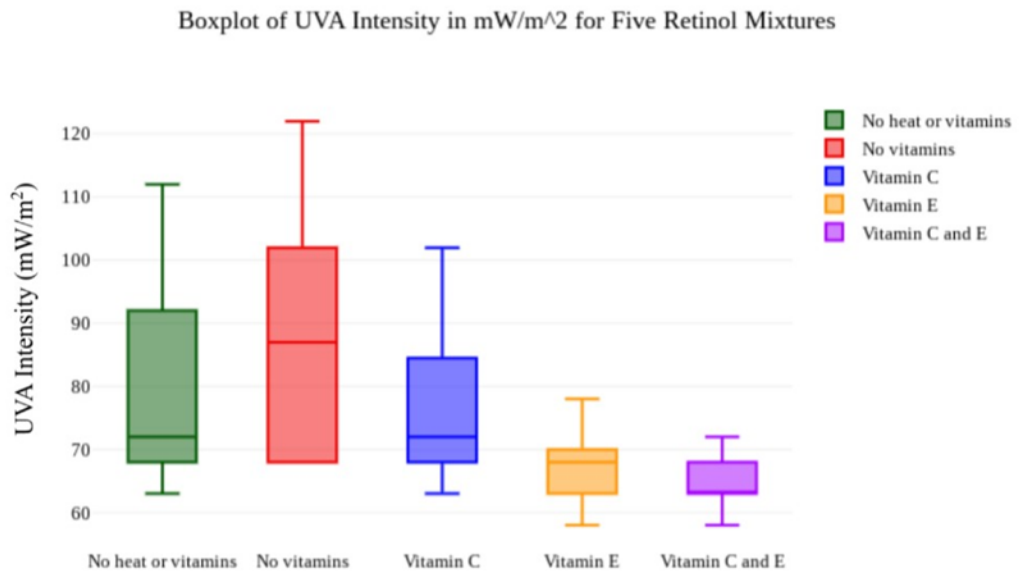

Figure 3. Boxplot Chart of UVA Intensity of All Five Retinol Mixtures *Note.* This boxplot depicts the minimum, first quartile, median, third quartile, and maximum of all five retinol groups.

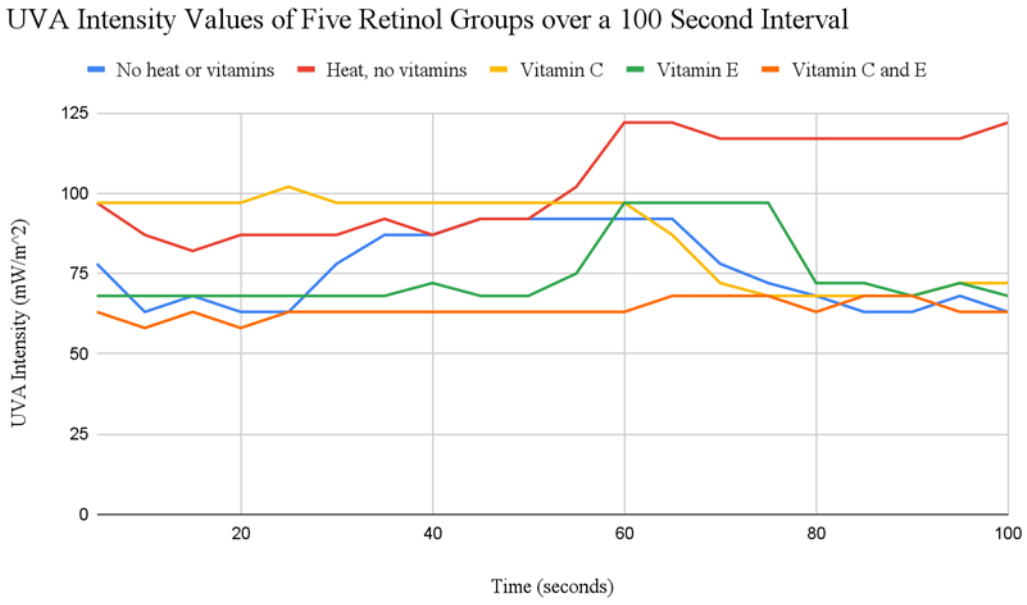

Figure 4. Line Graph of UVA Intensity Values (mW/m^2) over a 100 second period. *Note*. This graph depicts the UVA intensity values for one slide of each retinol group, or, one 100 second interval. In other words, one-third of the raw data for each retinol group is displayed, or 20 trials (one every 5 seconds).

Table 2. One-way ANOVA Test Summary Table (α= 0.05)

Source	*SS*	*df*	*MS*	*F*	*p*
Between	20428.120	4	5107.030	30.709	< 0.001
Within	49059.667	295	166.304		
Total	69487.787	299			

Note. This table depicts the calculated values for the one-way ANOVA test, at an alpha value of 0.05.

Table 3. *Post hoc Tukey test Summary Table*

Pair	Mean Difference	Critical Value	Statistically Significant?
No vitamins or heat vs. Heat, no vitamins	8.150	6.46	Yes
No vitamins or heat vs. Vitamin C	2.684		No
No vitamins or heat vs. Vitamin E	11.084		Yes
No vitamins or heat vs. Vitamin C and E	15.184		Yes
Heat, no vitamins vs. Vitamin C	10.834		Yes
Heat, no vitamins vs. Vitamin E	19.234		Yes
Heat, no vitamins vs. Vitamin C and E	23.334		Yes
Vitamin C vs. Vitamin E	8.400		Yes
Vitamin C vs. Vitamin C and E	12.500		Yes
Vitamin E vs. Vitamin C and E	4.100		No

Note. The table depicts the calculated mean differences between all of the five groups and the critical value, or Tukey value for the Post Hoc Tukey Test.

Appendix A

Table 4. Raw Data Table of UVA Intensity Values (mW/m^2) for Control Groups (retinol with no heat or vitamins, retinol with heat and no vitamins) and Experimental Groups (retinol with Vitamin C, retinol with Vitamin E, retinol with Vitamin C and E) https://rb.gy/damuv1