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Determining the impact of active ingredients on sunscreen UV resistance

Alex Chan
Department of Biomedical Engineering, University of Waterloo, 200 University Ave W., Waterloo, ON, Canada

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Abstract
In our continued efforts to eradicate skin cancer and other photodamaging effects caused by ultraviolet (UV) radiation, sunscreening agents have become our primary defense. However, despite the undeniable benefits of sunscreen, concerns have been raised due to the carcinogenic properties of several of their common active ingredients. Additionally, previous research has shown the unreliability of using sun protection factor (SPF) as a measure of sunscreen photoprotective ability. Given these considerations, this study set out to determine the impact different active ingredients would have on sunscreen UV resistance. It was hypothesized that the active ingredients' UV absorbance spectrum would provide a better predictor of sunscreen photoprotection. In this report, three different sunscreens were chosen – one with titanium dioxide, one with benzophenone derivatives, and one with salicylates – to provide a broad range of tested sunscreens. Due to their similarity with human DNA, *Saccharomyces cerevisiae* was cultured and used as a model system for these experiments. By comparing the percentage decrease of live cells protected with sunscreen relative to a control (i.e. no exposure to UV radiation), it was found that a titanium dioxide-based sunscreen was the most effective. Notably, all three sunscreens tested had an SPF 50, yet yielded different results in photoprotective ability.

Keywords: sunscreen, skin cancer, carcinogens, sun protection factor, solar ultraviolet radiation

1. Introduction

In our continued efforts to eradicate skin cancer and other photodamaging effects caused by UV radiation, sunscreening agents have become our primary defense. Though their beneficial effects have been proven by several scientific studies [1] the safety and efficacy of several sunscreening agents remain debatable [2]. Since 2004, research compiled by *Safe Cosmetics* – a coalition project funded by Breast Cancer Prevention Partners – has exposed the negative effects of common active ingredients used in sunscreening agents. Many of these ingredients, such as titanium dioxide, benzophenone and its derivatives, as well as homosalate, have been observed to display carcinogenic effects [3, 4, 5]. Considering the serious adverse effects potentially caused by the active ingredients used in sunscreens, testing the effectiveness and safety of sunscreens is of vital importance.

1.1 Sunscreen Related Indices: Sun Protection Factor (SPF)

The activity of a sunscreen is judged based on its SPF rating, which measures the sunscreen's capacity to block UVB radiation (there are currently no conventional measures for UVA radiation protection). SPF is calculated using the following equation:

$$SPF = \frac{MED\ of\ photoprotected\ skin\ (with\ sunscreen)}{MED\ of\ unprotected\ skin\ (without\ sunscreen)}$$

Where MED stands for minimal erythematic dose – essentially, the minimum UVB exposure time necessary to induce redness in the skin. Hence, if one's skin would normally redden after 10 minutes in the sun, sunscreen with SPF of 15 indicates that it would take 15 times as long (i.e. 2.5 hours) for the same effects to occur. Of course, these values are entirely theoretical as skin types – and hence melanin content and other biological factors – vary from person to person. In the United States, FDA regulations stipulate that a minimum of 10 human volunteers are tested and allows manufacturers to discard three of 10 test subjects [6]. Additionally, SPF is standardized to be measured with a sunscreen application density of 2 milligrams per centimeter squared [7]. Higher SPF values typically denotes higher efficacy, though this is not necessarily true in reality; according to the New Zealand Medical Journal, even after one hour of sun exposure, SPF could drop as much as 33% (depending on the formulation), and as much as 60% after swimming and active exercise [8]. In consideration of these details, reasonable doubt must be cast on the effectiveness of SPF as an index of a sunscreen's photoprotective ability.

1.2 Evaluating Different Sunscreening Agents

Depending on their mechanism of photoprotective action, sunscreening agents can be categorized as either topical or systemic sunscreens. Most sunscreens presently in commercial use are topical sunscreens, which are directly applied to the body to form a UV filter on the skin. Topical sunscreens can be further subdivided into inorganic or organic based sunscreens based on the nature of their active ingredients. Due to their widespread commercial availability, the sunscreens selected in this study were all topical sunscreens, representing the options commonly available to consumers. A total of three sunscreens were examined, one containing titanium dioxide (an inorganic sunscreen), another containing salicylate derivatives (an organic, primarily UVB-filter sunscreen), and a third containing benzophenone derivatives and avobenzone (an organic, primarily UVA-filter sunscreen). These three sunscreens were from

Ombrelle, Neutrogena, and SunBum respectively, and all had an SPF 50.

As previously mentioned, extensive research has shown the complications with relying on SPF as an index of sunscreen photoprotective ability. Instead, it is proposed that using the UV molecular absorbance spectrum of the active ingredients within the respective sunscreens would be a more reliable predictor of photoprotective ability.

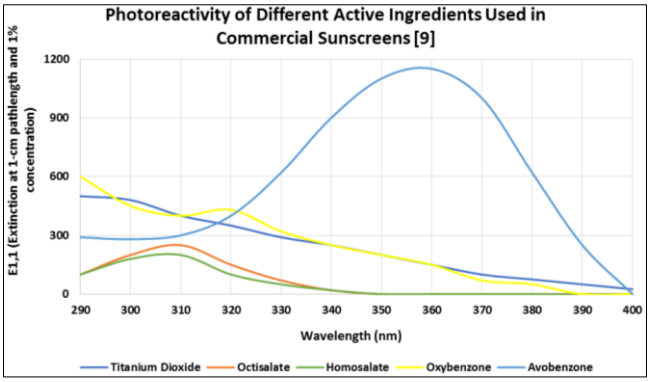


Fig 1: adapted from Jansen, *et al*., Photoprotection: Part II. Sunscreen: Development, efficacy, and controversies, illustrating the wavelengths of UV radiation protected by the active ingredients chosen in this experiment. The molar extinction coefficient (E) measures how strongly a chemical compound absorbs light at particular wavelengths, where more absorption would indicate better photoprotection.

Using Microsoft Excel 2019, the approximated areas under the curve were calculated and are summarized in the following table:

Table 1: Approximation of Area under for Molecular Absorption Curves

Titanium Dioxide	Octisalate & Homosalate	Oxybenzone & Avobenzone
21 075	13 400	93 850

As the active ingredients within the SunBum sunscreen have the greatest area under the curve, it is hypothesized that it will provide the most photoprotection, followed by the Ombrelle sunscreen.

2. Materials and Methods

**Procedure modified from the University of Utah, Genetic Science Learning Center* [10]

Saccharomyces cerevisiae (commonly known as baker's yeast), contains genes for DNA repair that are comparable to human genes with the same function [11]. Hence, baker's yeast was used as a model system to study the effects of UV radiation on cells. Exposure to UV radiation damages yeast cell DNA and can lead to cell death [12], enabling the observation of DNA damage by determining the percentage of live cells remaining after exposure to UV radiation. After applying the sunscreens to the lid of Petri dishes containing cultured yeast cells, they were exposed to UV light for 75 minutes. Using Adobe Photoshop and ImageJ photo processing software (Figure 2), the percentage area covered by live cells within a Petri dish was determined to compare the photoprotective ability of different sunscreens relative to the control (more live cells – and hence more area covered – will indicate a more effective sunscreen). From the measurements taken the mean and 95% confidence interval were calculated. Using graphing software, a bar graph was then plotted to compare the difference in live cells between the three sunscreens. An ANOVA test was also run, where $p < 5\%$ indicated a statistically significant difference.

2.1 Controls

To standardize the amount of yeast cells per individual trial, trials were organized per Petri dish. Each Petri dish was divided into four equal quadrants: a control group (no exposure to UV radiation), and the three remaining quadrants covered with sunscreen (i.e. SunBum, Ombrelle, and Neutrogena). Thus, trial 1 of SunBum, Ombrelle, and Neutrogena sunscreens were conducted on Petri dish 1, trial 2 was conducted on Petri dish 2, etc. This, along with multiple trials, would account for random error due to unconscious bias in streaking of Petri dishes with yeast cell solution. Additionally, temperature can affect the activity of the active ingredients within the sunscreens and must be kept constant. In this experiment, all trials were conducted at room temperature (~21℃) and yeast cell cultures were incubated at 25℃. To control the length of UV exposure, all trials were also conducted simultaneously and exposed for a period of 75 minutes. For this experiment, a blacklight with an emission spectrum between 280 – 400 nm and a peak emittance at 350 nm was used in place of sunlight. All sunscreens had a constant SPF of 50, thus any change in the efficacy of the sunscreen would be attributed to changes in active ingredients. Finally, the amount of sunscreen used was kept constant with FDA standards for the testing of sunscreen products – that is, 2mg/cm^2 of sunscreen [7].

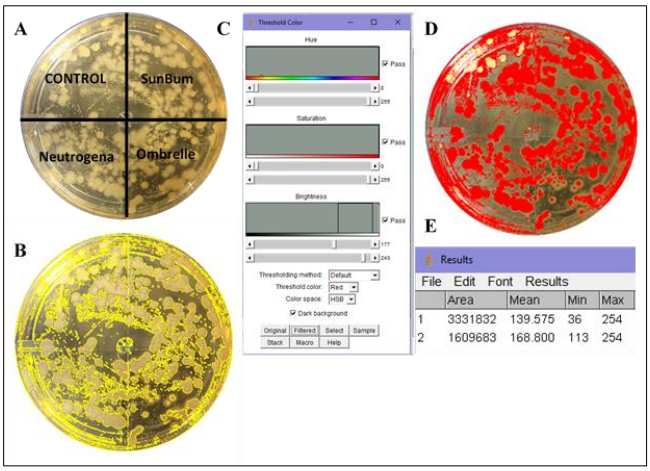
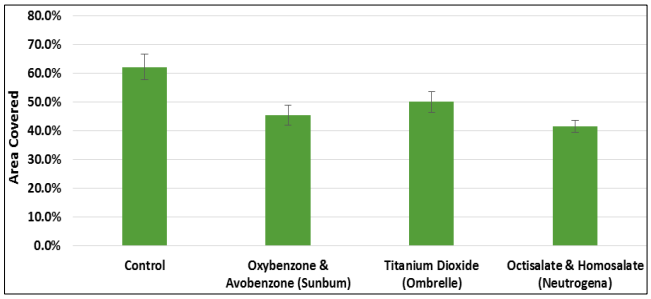


Fig 2: illustrates the procedure used to determine the live yeast cell coverage per trial. By dividing the Petri dish into quadrants, four trials were conducted per Petri dish (A). Using ImageJ, a threshold analysis was used to select the area covered by the yeast cells (i.e. the white spots) by adjusting the brightness filter (B and C, resulting in D). Given the area of live yeast cell coverage measured by pixels of the image, dividing by the total pixels of the image would give a total percentage area coverage (E, Graph 1). Similarly, this method can be used to determine the percentage decreases per each sunscreen sample relative to the control group – essentially, how effective the sunscreen was at protecting the yeast cells (Graph 2).

Results



Graph 1: Average Area Coverage of Live Yeast Cells for Different Sunscreens

Mean percent area coverage of live yeast cells and 95% confidence interval error bars for four independent sets of results, each of size $n = 10$. Successive confidence intervals are similar in length indicating limited variation due to error.

Graph 2: Effectiveness of Different Sunscreens Measured by Decrease in Live Yeast Cell Coverage Relative to Control

Mean percent decrease in area coverage of yeast cells relative to control group with no UV exposure. Ombrelle had the lowest decrease at 19%, indicating that it was the most effective sunscreen at protecting the yeast cells (least cell deaths). Error bars represent 95% confidence intervals for three independent trials, each of size $n = 10$.

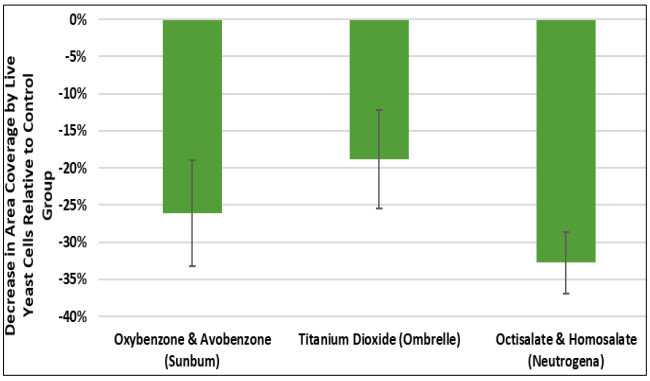


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Table 2: ANOVA Single Factor Results

Groups	Count	Sum	
SunBum	10	-2.60739	
Ombrelle	10	-1.88614	
Neutrogena	10	-3.27853	
Source of Variation	SS	dF	P-value
Between Groups	0.09698	2	0.014178
Within Groups	0.26167	27	
Total	0.358651	29	

Table 3: Post-Hoc Tukey HSD Results

Treatment Pairs	Tukey HSD Q Statistic	Tukey HSD P-value	Tukey HSD Inference
SunBum vs Ombrelle	2.14	0.30	Insignificant
SunBum vs Neutrogena	2.19	0.29	Insignificant
Ombrelle vs Neutrogena	4.45	0.01	Significant

4. Discussion

From the results collected in this experiment, it is apparent that a sunscreen's active ingredient affects its photoprotective ability and may have more bearing than its SPF. It was found that relative to the control, the Ombrelle sunscreen (titanium dioxide) performed the best with an average percent decrease in area coverage by live yeast cells of only 19%. The SunBum sunscreen (oxybenzone and avobenzone) performed second best with an average percent decrease of 26%. Finally, the Neutrogena sunscreen (homosalate and octisalate) performed the worst, with an average percent decrease in area coverage of 33%. It is important to note that all three sunscreens tested had an SPF of 50 yet yielded three different results in their ability to protect the yeast cells from UV radiation photodamage. From the post-hoc Tukey HSD results, it was found that only the Ombrelle sunscreen was statistically significantly better than the Neutrogena sunscreen, though the SunBum sunscreen also managed to outperform the Neutrogena sunscreen by quite a margin.

Hence, the hypothesis was partially disproven, as it was found that titanium dioxide provided better photoprotection than the formulation of homosalate and octisalate. As a tolerance of $p < 0.05$ was set for this experiment, other trends observed in the increased effectiveness of titanium dioxide relative to oxybenzone and avobenzone, and oxybenzone and avobenzone relative to homosalate and octisalate, approximated but failed to reach statistical significance. The results obtained in this experiment correspond with previous scientific research [9], as the two sunscreens with the active ingredients' that had the largest absorbance spectrum were the most effective. Though it was expected that the SunBum sunscreen would perform the best, there are two main reasons why the Ombrelle sunscreen could have outperformed it. Firstly, previous research has found that UVB rays are responsible for the most severe photodamage to our cells [13]. As avobenzone and oxybenzone are primarily UVA filters, despite their molecular absorption curves covering the largest area (Figure 1), titanium dioxide provides a lower magnitude of absorption but over a greater range (and thus encompassed protection from UVB radiation). Additionally, avobenzone has a relatively low photostability [14]. This means that exposure to UV radiation (i.e. irradiation) causes avobenzone to partially decompose, reducing its photoprotective ability while giving rise to potential phototoxic and photoallergic effects.

4.1 Limitations

This experiment had two potential limitations: using a threshold test as a method of analysis and the purity of the yeast cultures. The method of analysis was subjective as the threshold test to identify all white colors (i.e. the yeast cells) is dependent on the researcher. It is also difficult to determine potential error due to the use of imaging software. Though this may indicate that the data collected is not as reliable, it corresponds with previous

research on this subject [9, 13, 14]. Finally, though it was assumed that all cells that were cultured was *Saccharomyces cerevisiae*, the sample could have potentially been infected with a fungal or bacterial strain that is more resistant to UV radiation (e.g. mold) which was not accounted for in the results.

Considering these limitations, potential extensions for further research include: finding a more objective method of determining changes in yeast cell population relative to the control, as well as testing for bacterial growth to determine which percentage is actually yeast, and setting a tolerance on the percentage population that must be yeast for more accurate results. Additionally, these experiments were performed in vitro and thus would require in vivo validation. For example, salicylates are so commonly used due to their relative photostability [13] and may prove to be more effective than other active ingredients in vivo. Other active ingredients such as avobenzone may have unwanted biological effects due to reactions with the user's epidermal enzymes, thus reducing overall efficacy. It would be beneficial in the future to extend this experiment to other active ingredients to see if results continue to follow published data on the ingredients' absorbance spectrum.

5. Conclusion

Though it was hypothesized that the oxybenzone and avobenzone sunscreen would be the most effective due to the area under the ingredients' absorption curve, it was found that the sunscreen containing titanium dioxide significantly provided the best photoprotection. Additionally, the variability in the photoprotective ability of all three sunscreens in this study suggests that sunscreens' active ingredients greatly impacts a sunscreen's photoprotective ability despite identical SPF ratings. This implies that active ingredients' molecular absorption curves provide a more accurate estimate to a sunscreen's photoprotective ability than SPF and would ultimately provide better guidance to consumers in selecting safe and effective sunscreens

5. References

1. Gasparro FP, Mitchnick, M, Nash JF. A Review of Sunscreen Safety and Efficacy. Photochemistry and Photobiology. 1998; 68(3):243-256. doi:10.1111/j.1751-1097.1998.tb09677.x
2. Latha MS, *et al*. Sunscreening agents: a review. The Journal of clinical and aesthetic dermatology. 2013; 6(1):16-26.
3. Antony VB, *et al*. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans (Vol. 93) (World Health Organization, International Agency for Research on Cancer). Lyon, France: World Health Organization International Agency for Research on Cancer, 2010.
4. United States of America. California Environmental Protection Agency, Office of Environmental Health Hazard Assessment. Benzophenone, Coconut oil diethanolamine condensate (cocamide diethanolamine), Diethanolamine and 2-Methylimidazole Listed Effective June 22, 2012 as Known to the State to Cause Cancer. Sacramento, California: California Office of Environmental Health Hazard Assessment, 2012.
5. Jiménez-Díaz. I, *et al*. Simultaneous determination of the UV-filters benzyl salicylate, phenyl salicylate, octyl salicylate, homosalate, 3-(4-methylbenzylidene) camphor and 3-benzylidene camphor in human placental tissue by LC–MS/MS. Assessment of their in vitro endocrine activity. Journal of Chromatography B. 2013; 936:80-87. doi:10.1016/j.jchromb.2013.08.006
6. Paul SP. Ensuring the Safety of Sunscreens, and Their Efficacy in Preventing Skin Cancers: Challenges and Controversies for Clinicians, Formulators, and Regulators. Frontiers in Medicine, 2019, 6. doi:10.3389/fmed.2019.00195
7. United States of America, Food and Drug Administration, Center for Drug Evaluation and Research. Labeling and Effectiveness Testing: Sunscreen Drug Products for Over-The-Counter Human Use — Small Entity Compliance Guide. U.S. Food and Drug Administration, 2012.
8. Wilkins B. Sunscreens, 2017. Retrieved from https://www.nzma.org.nz/journal-articles/sunscreens
9. Jansen R, Osterwalder U, Wang SQ, Burnett M, Lim HW. Photoprotection Part II. Sunscreen: Development, efficacy, and controversies. Journal of the American Academy of Dermatology, 2013, 69(6). doi:10.1016/j.jaad.2013.08.022
10. Does Sunscreen Protect my DNA, 2002. Retrieved from https://teach.genetics.utah.edu/content/dna/Does-Sunscreen-Protect-My-DNA_TG.pdf
11. Pray LLH. Hartwell's yeast: A model organism for studying somatic mutations and cancer. Nature Education. 2008 1(1):183.
12. Takeshita K. Damage of yeast cells induced by pulsed light irradiation. International Journal of Food Microbiology, 2003 85(1-2)151-158. doi:10.1016/s0168-1605(02)00509-3
13. Abraham. A, Kaimal. S. Sunscreens. Indian Journal of Dermatology, Venereology, and Leprology. 2011; 77(2):238. doi:10.4103/0378-6323.77480
14. Paris C, Lhiaubet-Vallet V, Jiménez O, Trullas C, Miranda MÁ. A Blocked Diketo Form of Avobenzone: Photostability, Photosensitizing Properties and Triplet Quenching by a Triazine-derived UVB-filter. Photochemistry and Photobiology. 2009; 85(1):178-184. doi:10.1111/j.1751-1097.2008.00414.x