# Sunscreen Extraction and Evaluation

## Background Information

Excessive exposure to UV radiation is currently accepted as one of the main causes of skin cancer.1,2 Despite efforts towards educating the public on responsible Sun exposure habits and despite the wide availability of sun protective lotions, or sunscreens, the number of skin cancer incidence cases has been on the rise for the past few years.3-5 This may be related to the public’s misconceptions regarding the use of sunscreens; important factors such as amount and frequency of application, re-application after swimming or sweating and use of additional sun protection (hats, sun glasses, and clothing) are usually dismissed.

Since sunscreen lotions are the main form of sun protection used it is important that these products are efficient and safe. Sunscreen efficacy is usually evaluated in terms of sun protection factor (SPF), which is effectively a measure of the time taken for a detectable sunburn to occur in skin covered with 2 mg/cm2 of sunscreen when compared with untreated skin.6 SPF is related mainly to UVB protection but current regulations state that, for any given sunscreen, the UVA protection factor should be at least a third of the UVB protection factor.6 Moreover, a broad protection should be ensured and this is usually measured in terms of critical wavelength.6 This ensures that sunscreens not only absorb enough radiation, but also that absorption across the spectrum of harmful wavelengths is maximised.

The active ingredients in sunscreen lotions provide protection by absorbing the harmful UV radiation instead of the skin. Absorption of UV/Vis radiation by any molecule will excite its electrons to a higher energy electronic state, and the molecule is then said to be excited, or in an excited state.7 An ideal sunscreen molecule will dissipate the excess energy quickly (roughly within picoseconds, 10-12 s) and without undergoing or prompting any potentially harmful side chemistry, such as the release of free radicals. The persistence of excited (*i.e.* reactive) states in the skin for long periods of time (in the order of seconds) increases the chance for undesirable side reactions to occur between different components of the sunscreen formulation or between the sunscreen ingredients and the skin.



In this experiment, you will have the chance to evaluate the efficacy of different brands of sunscreens and their respective SPF values by comparing UV/Vis absorbance spectra. You will also collect fluorescence data of sunscreen active ingredients in order to evaluate the existence of potentially harmful long lived excited states.

**References**

1. D. L. Narayanan et al., Int. J. Dermatol., 49, 2010.
2. D. S. Rigel, J. Am. Acad. Dermatol., 58, 2008.
3. R. L. Siegel, K. D. Miller, A. Jemal, CA Cancer J. Clin., 66, 2016.
4. World Health Organization, *Health statistics and information systems: Mortality database*, 2015.
5. Cancer Research UK, Skin Cancer, 2013.
6. G. Verheugen, *Commission recommendation on the efficacy of sunscreen products and the claims made relating thereto,* Brussels, 2006.
7. P. Atkins and J. de Paula, *Physical Chemistry*, 10th Edition, OUP Oxford, 2014.

## Learning Outcomes

* Comparing sunscreen brands and SPF values in order to identify possible weaknesses with the SPF method for evaluating sunscreen efficacy.
* Becoming familiar with photochemical and photophysical processes, in particular fluorescence.
* Becoming familiar with fluorometry and the concept of time-resolved spectroscopy.
* Data analysis and presentation.
* Critical thinking.

## Safety Information

**Methyl 4-methoxycinnamate (C11H12O3, MMC)**

Harmful by inhalation, in contact with skin, and if swallowed. In case of exposure immediately wash contact area with copious amounts of running water for at least 15min. If inhaled remove to fresh air. If ingested wash mount with copious amounts of water and seek medical attention. If spilled mix with sand or similar inert absorbent material, sweep up and keep in tight container for disposal.

**Methyl anthranilate (C8H9NO2, MA)**

Causes eye irritation. If in eyes, rinse cautiously with copious amounts of water.

**Isopropyl Alcohol (C3H8O)**

Hazardous in case of eye contact (irritant), of ingestion, of inhalation. Slightly hazardous in case of skin contact (irritant, sensitizer, permeator). In case of exposure immediately wash contact area with copious amounts of running water for at least 15min. If inhaled remove to fresh air. If ingested, seek medical advice immediately and show the container or the label. DO NOT induce vomiting.

**Cyclohexane (C6H12)**

Hazardous in case of skin or eye contact, ingestion or inhalation. In case of exposure immediately wash contact area with copious amounts of running water for at least 15min. If inhaled remove to fresh air. If ingested, seek medical advice immediately and show the container or the label. DO NOT induce vomiting.

**UV LED’s (UV-B radiation)**

UV light, even at the low intensities used in this experiment, can be detrimental to skin and vision hence exposure is to be avoided. The UV light source will be contained within a sealed, opaque black box which completely blocks the light. The fluorometers will be built such that emission of UV radiation is only possible when this box is securely closed.

## Practical Technique

## Before the Experiment

Read the experimental method carefully. Complete your pre-lab tasks. Albeit not compulsory, you might consider reading the background information references to better prepare for this experiment. Also, consider the following resources:

* Fluorescence spectroscopy *or* spectrofluorometry (mainly up to 02:10): <https://www.youtube.com/watch?v=eWXsX2xpaQk>
* Time-resolved fluorescence spectroscopy (also check related topics on left column of this web page): https://www.edinst.com/techniques/time-resolved-fluorescence/

## Experimental Method

**Sunscreen brands and SPF**

Two different brands of sunscreens will be used, A and B. For each brand, four different SPF values will be analysed, *i.e.* A(0), A(15), A(30), A(50) and corresponding for B. The sample with an SPF value of zero corresponds to a solar product by the same brand that does not provide sun protection, i.e. an after sun lotion. You are responsible for keeping track of your multiple samples by ensuring appropriate labelling and record keeping.

* Combine 1g of each sunscreen with 50 ml isopropyl alcohol in a polypropylene centrifuge tube.
* Shake the mixture to suspend the sunscreen and then heat in a water bath to 45-50°C for 1min.
* Cool the mixture to room temperature and then centrifuge for ~5min.
* Collect the supernatant for further analysis.
* Collect a UV/Vis spectrum of each sample and plot the data to complete post-lab task 1.

**Photophysics of sunscreen active ingredients**

Common sunscreen active ingredients usually fall into one of seven categories (see below): *para*-aminobenzoate, cinnamate, salicylate, anthranilate, camphor, dibenzoyl methane and benzophenone derivatives. Two examples of these will be used the next step of the experiment: methyl-4-methoxycinnamate (MMC) and menthyl anthranilate (MA).

* Prepare two separate solutions of MMC and MA in cyclohexane to a concentration of 3mM.
* Take a UV/Vis spectrum of each of these samples.
* Take your samples to the fluorometer; your demonstrator will talk through how it works.
* Taking into account the UV/Vis spectra you collected for your MMC and MA samples, select the correct LED wavelength emission to be used in your fluorescence measurements.
* Record the fluorescence signal from your samples and discuss what the implications of your results are in the context of using MMC or MA as sunscreen active ingredients.
* You will be given data from time-resolved fluorescence measurements on these molecules. While your demonstrator will cover this with you, also refer to <https://www.edinst.com/techniques/time-resolved-fluorescence/> for more details on this technique.
* Fit these data and extract fluorescence lifetimes.

When you have finished make sure all glassware you use is washed and dried, and any quartz cuvettes are rinsed thoroughly with acetone and dried.



*Categories of sunscreens: (a) aminobenzoates, (b) cinnamates, (c) anthranilates, (d) salicylates, (e) camphor derivatives, (f) dibenzoyl methane derivatives and (g) benzophenone derivates. Categorised as in F. P. Gasparro, Sunscreen Photobiology, New York, Springer, 1997.*

## Further Reading

If you are interested in knowing more about the material covered in this experiment, please refer to the references for the background information and/or:

* About sunscreens: F. P. Gasparro, *Sunscreen Photobiology*, New York, Springer, 1997.
* About spectroscopy: J. M. Hollas, *Modern Spectroscopy*, England, John Wiley & Sons Ltd, 2004.
* About ultrafast time-resolved spectroscopy: A. Zewail, *J. Phys. Chem. A*, **104(24)**, 2000.
* About sunscreens studied with ultrafast spectroscopy techniques (from work currently being done at Warwick): L. Baker *et al.*, Science Progress, **99(3)**, 2016, 282-311 and N. D. N. Rodrigues *et al.*, *Proceedings of the Royal Society A*, **472**, 2016.

**Pre-Lab Questions**

1. What safety considerations must you keep in mind throughout this experiment?
   1. Wear gloves, lab coat and protective goggles
   2. Always use solvents in a fumehood and never pour them down the sink.
   3. Be careful not to leave fingerprints on the clear sides of quartz cuvettes.
   4. All of the above.
2. What wavelengths correspond to UVA and UVB?
   1. UVA: 315-280 nm; UVB: 280-100 nm
   2. UVA: 400-700 nm; UVB: 700-1000 nm
   3. UVA: 400-315 nm; 315-280 nm
   4. They are different ways to say the same thing.
3. What type of energy levels are accessed upon absorption of UV/Vis radiation?
   1. Vibrational
   2. Rotational
   3. Electronic
   4. All of the above
4. What type of function describes an excited state decay?
   1. Polynomial
   2. Linear
   3. Exponential
   4. Logarithmic
5. Luminescence is a term that encompasses all radiative photophysical decay processes, that is:
   1. Fluorescence and internal conversion
   2. Fluorescence and phosphorescence
   3. Fluorescence, phosphorescence and intersystem crossing
   4. Intersystem crossing and internal conversion
6. What information does a time-resolved study provide that cannot be obtained by static measurements (such as standard UV/Vis or fluorescence spectroscopies)?
   1. No further information is obtained, it just provides better data.
   2. The evolution of a system with time can be monitored with time-resolved techniques and hence these studies yield information on dynamics occurring upon absorption of radiation by a molecule.
   3. Time-resolved spectroscopy techniques, unlike static techniques, have an element of imaging and hence provide spatial information.
   4. Time-resolved techniques allow for the rate of reactions to be controlled, hence the variable of time is taken out of the equation and can be assumed not to interfere with results and conclusions.

**Post-Lab Tasks: Assignment**

1. **Plot your UV/Vis data clearly demonstrating the relationship between SPF value and absorbance within the same brand. Compare and comment on sunscreen efficacy (absorbance) with respect to (a) different SPF values and (b) different brands.** Think about how many graphs you need, what your axes will be and what scale you will use. Always remember to be concise, clear and to keep a professional presentation of data throughout.
2. **Draw a simplified Jablonski diagram which clearly shows five photophysical processes that may occur upon absorption of UV/Vis radiation. Mark the ones that you suggest may be happening in the molecules studied, supporting your decisions with experimental data.** You may find your notes on the Introduction to Physical Chemistry module a useful aid for this exercise (and the ones below).
3. **Present the plots of the time-resolved fluorescence data you were provided with, along with fitted curves and time constants/lifetimes extracted. Also give the rate constants associated with the excited state decays for each molecule.** Please ensure that you start this within lab time as you may need support from your demonstrator. Once again refer to your notes on Introduction to Physical Chemistry.
4. **Considering the background information with which you were provided, comment on the suitability of MMC and MA as sunscreen active ingredients.** Particularly, think about ideal decay timescales for sunscreen molecules, the typical timescales of the decay processes likely to be happening in these molecules, and the implications of that towards the persistence of excited states in the sunscreen mixture. Refer to the background information for some more information.

**Post-Lab Tasks: Mark Scheme and Answers**

**In lab practical component: Pass/Warning/Fail**

The student passes if they show awareness of safety considerations, competence in practical skills and responsibility for good general lab practice. Any problems or deviations from ideal lab performance should be flagged as warnings. Anything below minimum standard of appropriate lab behaviour should be considered a fail.

**Assignment**

1. **Plot your UV/Vis data clearly demonstrating the relationship between SPF value and absorbance within the same brand. Compare and comment on sunscreen efficacy (absorbance) with respect to (a) different SPF values and (b) different brands.**

Looking for the student to reflect on what’s the best way to display the data to demonstrate a certain relationship. For (a) ideally looking for two plots, one for each brand, displaying all UV/Vis spectra collected for the different SPF value samples of each brand. In (b), ideal case would be to have a plot of peak absorbance vs SPF with a trace for each brand. Other formats can be accepted.

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| **Task 1** | |
| 3/3 | Data is presented clearly, concisely and professionally. Presentation of data clearly and unambiguously shows (a) SPF-absorbance relationship and (b) difference between same SPF in different brands. Correctly comments on the effects of increased SPF on absorbance and compares different brands, noting any changes in absorbance for same SPF value. Adds comments regarding the broadness of UV/Vis absorption as another important factor for photoprotection. |
| 2/3 | Misses showing relationship (a) or (b) correctly but comments on results are satisfactory *OR* Clearly shows relationship (a) and (b) but comments on results are not satisfactory. |
| 1/3 | Misses showing relationship (a) and (b) but comments are satisfactory *OR* Clearly relationship (a) or (b) but comments are not satisfactory. |
| 0/3 | No attempt at the above or all incorrect. |
| Notes | Half marks can be awarded at demonstrator’s discretion; detailed feedback must be provided to the student regardless. |

1. **Draw a simplified Jablonski diagram which clearly shows five photophysical processes that may occur upon absorption of UV/Vis radiation. Mark the ones that you suggest may be happening in the molecules studied, supporting your decisions with experimental data.**

The student should be able to recognise that fluorescence and/or phosphorescence is occurring in these molecules (hence why fluorescence signal is observed). They should also recognise that intersystem crossing needs to occur for phosphorescence to be possible.

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| **Task 2** | |
| 2/2 | Correctly draws a Jablonski diagram and identifies five photophysical processes (internal conversion, intersystem crossing, intramolecular vibration redistribution, fluorescence, phosphorescence or others if correct). Is able to identify that, for the molecules where fluorescence is measured, that is the process occurring; where no fluorescence is measured, any of the other photophysical processes could be occurring. |
| 1/2 | Draws Jablonski diagram and identifies five photophysical processes but fails to make connection to experimental results. |
| 0/2 | No attempt at the above or all incorrect. |
| Notes | Half marks can be awarded at demonstrator’s discretion; detailed feedback must be provided to the student regardless. |

1. **Present the plots of the time-resolved fluorescence data you were provided with, along with fitted curves and time constants/lifetimes extracted. Assuming these lifetimes correspond to the total decay from all possible processes, also give the rate constants associated with the excited state decays for each molecule.**

The student should present clear, professional plots of the data provided, with a clear kinetic fit line. Lifetimes extracted should be clearly presented, ideally with associated errors. Students should also be able to recognise that the rate constant is the reciprocal of the lifetime.

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| **Task 3** | |
| 2/2 | Presents clear, professional plots of data provided, along with a kinetic fit line and clear indication of extracted lifetimes, with appropriate units. Correctly calculates |
| 1/2 | Presents appropriate plots but does not or incorrectly calculates rate constant. OR Does not present appropriate plot (not up-to-standard or no kinetic fit) but correctly calculated rate constant. |
| 0/2 | No attempt at the above or all incorrect. |
| Notes | Half marks can be awarded at demonstrator’s discretion; detailed feedback must be provided to the student regardless. |

1. **Considering the background information with which you were provided, comment on the suitability of MMC and MA, respectively, as sunscreen active ingredients.**

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| **Task 4** | |
| 3/3 | Recognises that an effective sunscreen needs to dissipate energy quickly and hence fluorescence is not ideal due to its typically long lifetime. With this in mind, non-fluorescent MMC should be pointed out as a good candidate for sunscreen use, while the strongly fluorescent MA should be pointed out as a non-ideal sunscreen molecule. Clearly constructs the argument and presents conclusions. |
| 2/3 | Shows some evidence of understanding the background information. Struggles to justify suitability of molecules under study for sunscreen mixtures or does so incorrectly. |
| 1/3 | Mentions background information on the ideal characteristics of a sunscreen but fails to make the connection to the experimental data. |
| 0/3 | No attempt at the above or all incorrect. |
| Notes | Half marks can be awarded at demonstrator’s discretion; detailed feedback must be provided to the student regardless. |