Investigating the synergy in the battle against UV between sea and plant-based extracts for bio-inspired cosmetics applications

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Abstract

Background: Molecules from nature, such as mycosporine-like amino acids (MAAs) from aquatic environments and sinapoyl malate (SM) from the plant kingdom have individually been shown to have beneficial cosmetic properties. However, it is currently unknown whether combining these two ingredients can work together to enhance their favourable qualities with a synergistic effect.

Methods: Commercial forms and isolated dried extracts of products containing SM - garden cress sprout extract (*Lepidium sativum*), and MAAs - red Nori algae extract (*Porphyra umbilicalis*) - were blended in different combinations. Each combination was studied using state-of-the-art ultrafast laser spectroscopy (ULS) to track the energy flow within photoactive compounds in real-time. Additional solar irradiation studies that analyse changes in the UV absorption profiles of the ingredients over time, combine to offer a detailed picture of synergistic effects.

Results: Excellent UV photostability (less than 10% reduction for the commercial forms) was demonstrated in irradiation tests using a solar lamp over two hours. Higher photostability was observed when the two ingredients were combined, providing evidence of a synergy between the two ingredients ULS measurements rationalise these observations in more detail.

Conclusions: Combined, these techniques shows the potential of fine-tuning the ratios of these nature-based ingredients for maximum cosmetic effect. Biomimicry from both the land and the sea can be blended in harmony to enhance cosmetic properties. This could incorporate both properties which they have already been proven to possess, whilst adding the potential of new activities, such as UV boosting.

Keywords: Nature-inspired; Photochemistry; Mycosporines; Sinapoyl malate

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Conflicts of Interest: There are no conflicts of interest to declare.

Introduction

Active ingredients inspired by nature are increasingly being developed and incorporated into cosmetics and beauty products. Molecules from nature, such as mycosporine-like amino acids (MAAs) from aquatic environments and sinapoyl malate (SM) from the plant kingdom, have individually been shown

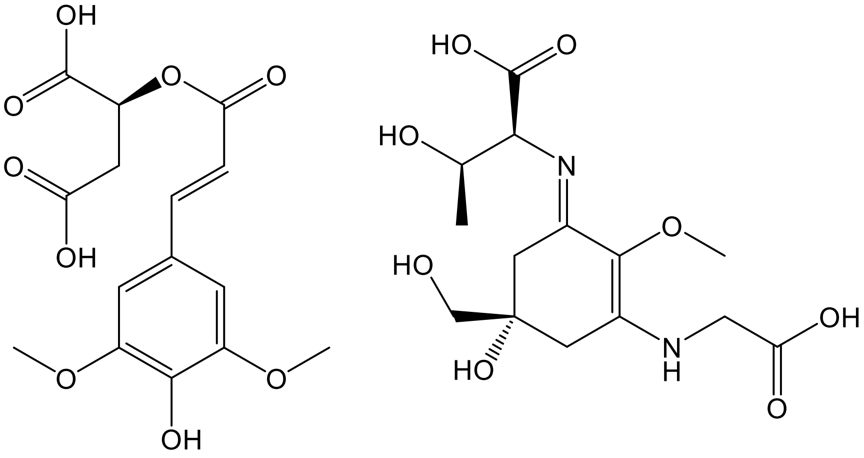

Fig. 1: Chemical structures of **(left) sinapoyl L-malate**, a molecule contained within Detoxophane nc; and **(right) porphyra-334**, a mycosporine-like amino acids extracted from Noriguard nc and Helioguard™ 365. Structures based upon those presented in refs. (5) and (12).

to have beneficial cosmetic properties, including anti-ageing, antioxidant and UV absorption. (1-5) The chemical structures of SM and porphyra-334, an MAA, are shown in **Fig. 1** for reference.

A previous collaboration between the University of Warwick and Mibelle Group Biochemistry found that both Detoxophane nc (*Lepidium Sativum* Sprout Extract with a lecithin encapsulation) and its constituent dried garden cress sprout extract had potential as a UV booster, and that the extract contained a high concentration of SM. (6) For MAAs, previous studies at the University of Warwick have shown that they have an excellent photostability profile and promising characteristics for UV filtration. (7-9) The MAAs studied include shinorine and porphyra-334 extracted from Helioguard™ 365 (*Porphyra Umbilicalis* extract, lecithin encapsulation), a Mibelle Group Biochemistry commercial product. (9) The name porphyra-334 arises from the peak absorption wavelength of this molecule, which is 334 nm.

Despite these ingredients being studied in detail individually, it is unknown whether combining ingredients from different natural sources can work together to enhance their favourable qualities with a synergistic effect, as opposed to solely an additive effect observed previously for inorganic UV filters. (10) To this end, this collaboration has sought to investigate the effect of combining Detoxophane nc with Noriguard nc. Noriguard nc is a simplified version of Helioguard™ 365 containing between 0.08% and 0.15% of MAAs, with their novel dried extracts sourced from nature and not encapsulated. If synergies between these two ingredients can be identified, this may alleviate some of the challenges associated with low extracted yields of natural active ingredients, as the power of individual components could be increased and small quantities of these would suffice. Furthermore, the concentrations of synthetic UV filters in a formulation could be reduced in a final product if the nature-based ingredients can boost their activity. This would benefit both formulators and customers alike. (11)

The specialism of the research team at Warwick, implemented throughout this study, is ultrafast transient electronic absorption spectroscopy (herein abbreviated as TEAS). In recent years, TEAS studies have been extensively tried and tested to identify the photoprotective properties of MAAs and sinapoyl derivatives. (12) Its applications to sunscreens were presented previously at IFSCC 2018, which was the basis for a recent publication on avobenzone photostability. (13) Fundamentally, the technique employs pairs of laser light pulses, which traverse through the sample. The first laser pulse is called the "pump" pulse, which photoexcites the UV absorbers in solution, *i.e.* it promotes these molecules from their ground electronic state to higher energy (excited) electronic states. The pump pulse mimics the action of solar UV radiation upon interactions with chromophores in the sample. In general, the excitation wavelength is chosen to be its UV-visible absorption maximum ($\lambda_{\max}$), which is usually determined by steady-state UV-visible spectroscopy. (12) Following the pump step, the "probe" pulse interacts with the sample molecule, to observe the relaxation of the molecules following photoexcitation. This is monitored by assessing the difference in light transmitted through the pumped (photoexcited) and unpumped (not photoexcited) samples. The resulting data is presented in units of ΔOD, "change in optical density", which is calculated by subtracting the logarithm of the signal recorded from the photoexcited sample (I_p), from the equivalent value for the non-photoexcited sample (I_0); equivalently represented in Equation 1.

$$\Delta OD = \log\left(\frac{I_0}{I_p}\right) = \log I_0 - \log I_p \tag{1}$$

The probe pulse is delayed in time (referred to as a time delay, Δt) in increments typically between zero and 2 nanoseconds (ns, where 1 ns = 1 x 10^{-9} seconds). By varying the value of Δt, photochemical changes following photoexcitation can be observed over time, from the instant that radiation is absorbed. Transients collected in time intervals between $0 \leq \Delta t \leq 2$ ns are collated to give a relaxation profile of the molecule as it evolves, *i.e.*, how the molecule deals with the excitation energy immediately after it has been absorbed. This information can then be used to understand the long-term behaviours of photoexcited molecules. The "ultrafast" portion of the technique's name refers to the intrinsic femtosecond (10^{-15} s) time-resolution of the technique. The instrument response of 80 – 140 fs that we achieve facilitates observation of the decay of intermediate species that may be invisible to other analytical methods, such as laser flash photolysis, which uses pulses that are not narrow enough in time to capture short-lived transient species. Due to the complex nature of the TEAS setup, a full description is omitted in this report. Other references detail the specifics of the experimental setup used for these measurements, should the reader be interested. (14, 15) TEAS has been used to investigate a wide range of cosmetics applications, such as UV filters, antioxidants and blue-light absorbers.

Materials and Methods

Commercial forms and isolated dried extracts of products containing garden cress sprout extract (*Lepidium sativum*) and red Nori algae extract (*Porphyra umbilicalis*) were provided by Mibelle Group Biochemistry (Buchs, Switzerland). These ingredients were then blended in different combinations to investigate whether they enhance the activity of one-another. Full details of the samples used to attain the data in the figures presented will be given in the relevant figure caption. In general, the commercial forms of both Detoxophane nc and Noriguard nc required 10-fold and 20-fold dilution with deionised water, respectively, to ensure an appropriate absorbance suitable for UV-visible spectroscopy measurements (< 1). Commercial products were used as provided for TEAS measurements, dilution was not required. Where mixtures were needed, the necessary ratios were combined by volume, then gently shaken to ensure a good blend. Around 15 – 20 mL total volume was required for each set of measurements.

For UV-visible spectroscopy of the dried extracts, around 0.1 mg/mL of each extract was dissolved in deionised water to attain a suitable absorbance. Solubilization was possible by gentle shaking/magnetic stirring. For TEAS measurements of dried extracts of *Lepidium sativum* sprout and *Porphyra umbilicalis*, samples was passed through a 0.2 μm filter fitted to a syringe containing the sample to sterilise it, to mitigate the risk of bacterial contamination. The dried extracts were proven to be very soluble in water, as the absorbance was equivalent before and after sterilisation with the filter.

The relevant details regarding the instrumentation for this work are as follows: for TEAS measurements, photoexcitation was achieved using a pump pulse of 330 nm (the peak absorption of the MAAs in Noriguard nc and SM), with a pulse power of 0.5 mW at the sample. The probe pulse was a broadband white-light continuum spanning the spectral range of 330 – 675 nm. Transients were taken at pre-defined time delays between $\Delta t = 0$ and 1.9 ns unless stated otherwise. The path length was set to either 100 μm or 950 μm, depending on the experimental requirements. The specifics for each experiment are provided in the relevant figure caption. For steady-state UV-visible spectroscopy measurements, a Cary-60 instrument (Agilent Technologies) was used. All UV-visible spectra were taken in a quartz cuvette with a path length of 10 mm. Samples were irradiated using a Newport Oriel 94011A solar simulator, contained within these same cuvettes.

Results

Firstly, the UV absorption profiles of the two commercial products are presented in Fig. 2. These results reveal that Detoxophane nc absorbs mainly in the UVB region, whereas Noriguard nc predominantly absorbs UVA radiation. In previous studies, Noriguard nc has been found to have a low *in vitro* SPF value, as it only absorbs a very small proportion of UVB radiation, the main determining factor for SPF. Detoxophane nc alone has also been assigned a low *in vitro* SPF value, as its overall absorbance is relatively low, although there is plenty of scope for this to be improved via a synergetic effect.

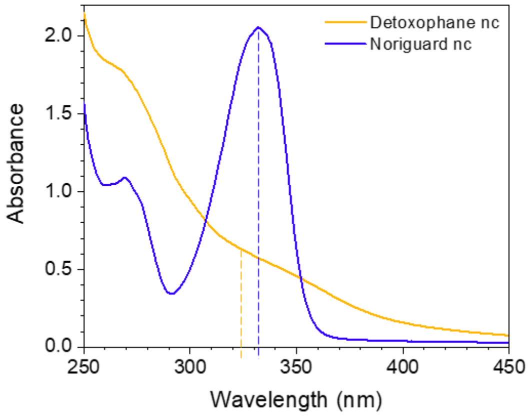

Fig. 2: (Yellow line) UV absorption spectrum of Detoxophane nc (10-fold dilute in deionised water). The dotted yellow line is indicative of the peak absorption wavelength (λ_{max}) of sinapoyl malate, which has previously been shown to be contained in this ingredient. (Blue line) UV absorption spectrum of Noriguard nc (20-fold dilute in deionised water). The blue dotted line is the λ_{max} value of Noriguard nc (~332 nm).

Fig. 3 shows the UV absorption profiles of the Detoxophane nc and Noriguard nc alone (diluted) after exposure to solar simulated radiation for two hours. During this time, both commercial forms demonstrated a reduction in absorbance of 6%. These values were found by integrating the area under the curve before and after irradiation, ± 5 nm from the central wavelength. Also presented in Fig. 3 are the results for 1:1 combinations of the commercial forms and the dried extracts in deionised water and irradiated under the same conditions. For clarity, the percentage reductions are summarised in Table 1.

The 1:1 combination of the commercial forms showed higher photostability than when they were separate, 2% compared to 6%, as observed when comparing the results presented in Fig. 3. This is the first evidence of a positive synergetic effect between the two ingredients. This data also shows that the commercial forms degraded less over two hours than the dried extracts in water, showing percentage decreases of 2% and 10% respectively. The measurements in Fig. 3 and Table 1 were repeated on two occasions, with the same trends in the results shown both times. The only exclusion is the dried extracts in combination, due to limited sample availability for a repeat measurement.

Once the steady-state properties and potential synergies between Detoxophane nc, Noriguard nc and the dried extracts had been established, ultrafast spectroscopy investigations using transient electronic absorption spectroscopy (TEAS) were conducted, using the methodology described in the Introduction and Methods sections. This was to study changes to the excited-state dynamics of photoactive molecules between different combinations of ingredients, which could be indicative of potential synergies.

For the TEAS data to follow, spectra are presented two-dimensionally in the form of a colour map (a type of contour plot), where the colour at a given point indicates signal magnitude for that wavelength

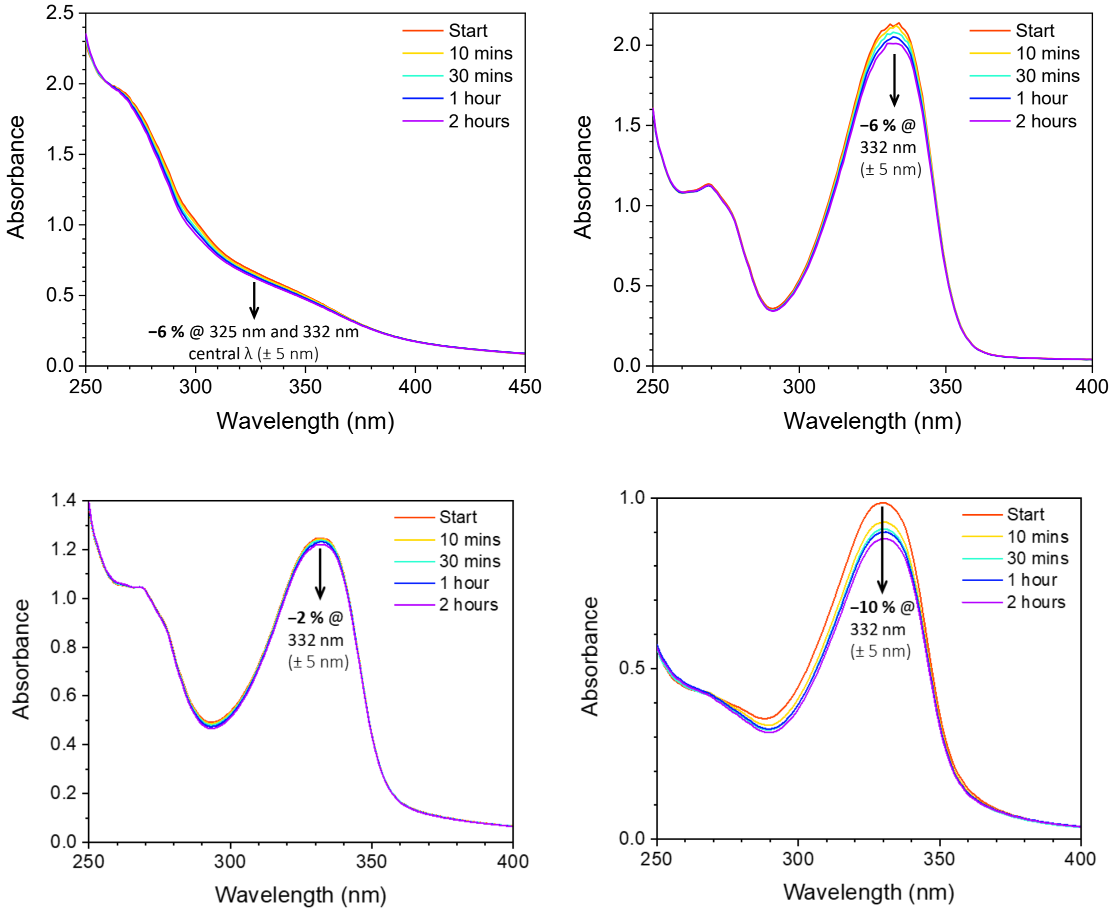

Fig. 3: Top row: (Left) UV absorption spectra of Detoxophane nc (10-fold dilute in deionised water); (Right) Noriguard nc (20-fold dilute in deionised water) after between 0 – 2 hours of irradiation using a solar simulator. Bottom row: (Left) UV absorption spectra of the commercial forms of Detoxophane nc and Noriguard nc, combined in a 1:1 ratio by volume, and diluted with deionised water. The reduction in absorbance was calculated at the peak wavelength of 332 ± 5 nm; (Right) UV absorption spectra of the dried garden cress sprout extract and *Porphyra umbilicalis* extract, combined in deionised water in a 1:1 ratio by mass (0.1 mg/mL of each). The reduction in absorbance was calculated at the peak wavelength of 332 ± 5 nm.

and time delay (Δt). In addition, mΔOD traces for specific values of Δt, as a function of wavelength, are presented alongside the colour maps. These traces, known as "transients",[1] contain information regarding the photophysical processes[2] occurring at time Δt. (16) If these processes can be assigned, then the reasons for potential synergies between the ingredients can be investigated.

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[1] A convolution of positive ΔOD signals is indicative of excited state absorption or photoproduct absorption. Negative signals suggest stimulated emission, which can be thought of as laser-induced fluorescence or ground state bleaching. (12)

[2] These processes can be radiative, *e.g.* fluorescence and phosphorescence; or non-radiative, *e.g.* vibrational cooling, internal conversion and intersystem crossing.

Table 1: Summary of the percentage UV decrease in each of the four samples tested, for the data shown in Figs. 2 and 3.

Sample	% decrease
Detoxophane nc only (diluted 10-fold)	6
Noriguard nc only (diluted 20-fold)	6
Detoxophane nc, Noriguard nc combined 1:1	2
Dried garden cress extract alone (ref. 6)	18
Dried garden cress extract and Nori extract (1:1 by mass)	10

Studies on the garden cress (*Lepidium sativum*) sprout extract alone were conducted previously in an earlier Mibelle Group Biochemistry/Warwick University collaboration (6), however, the ultrafast characteristics of Noriguard nc alone had not yet been identified. Therefore, Noriguard nc alone was the starting point for this study, with the results shown in Fig. 4. Previous work by Whittock *et al.* (9) on the MAAs contained within Helioguard™ 365, reveals the same spectral features as those seen here for Noriguard nc. The key features of this data are that the amount of signal decreases rapidly, suggesting that the molecule returns to its lowest energy ground-state quickly, with no adverse effects such as radiative decay or photodegradation. This is positive for cosmetics applications as harmful reactions on the skin are less likely. From the data in Fig. 4, it can be concluded that the components surrounding the MAAs in Noriguard nc are not impacting the excited-state relaxation pathways following UV photoexcitation.

Once this was established, Detoxophane nc and Noriguard nc were combined: firstly, to determine the effect of the addition of this ingredient, and secondly, to observe potential reasons for the synergies observed in the steady-state and in vitro SPF measurements. Fig. 5 shows the results when Detoxophane

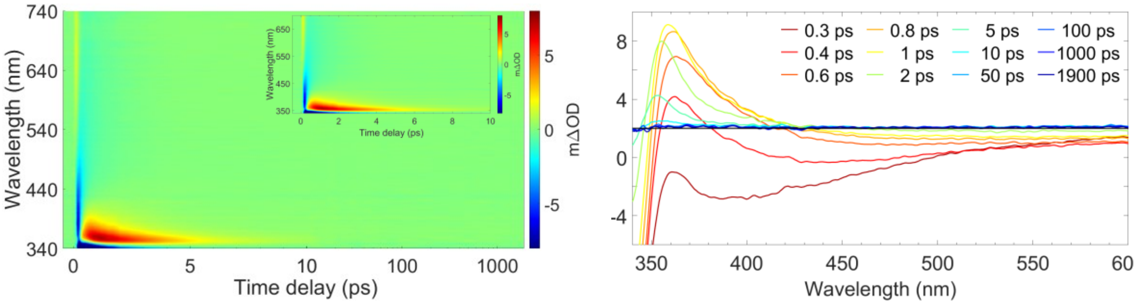

Fig. 4: TEAS spectra of the undiluted commercial form of Noriguard nc, photoexcited at 330 nm with 100 μm path length. (Left) False colour heat map showing all transient absorption spectra (TAS) of Noriguard nc only. The time delay axis is linear up to 10 ps and logarithmic thereafter. Inset is a zoom-in of the TAS up to 10 ps, presented on a linear scale. (Right) TAS at selected pump-probe time delays: these plots are attained by taking vertical slices through the false colour heat maps at the given time delay and are presented on the same scale as the heat map.

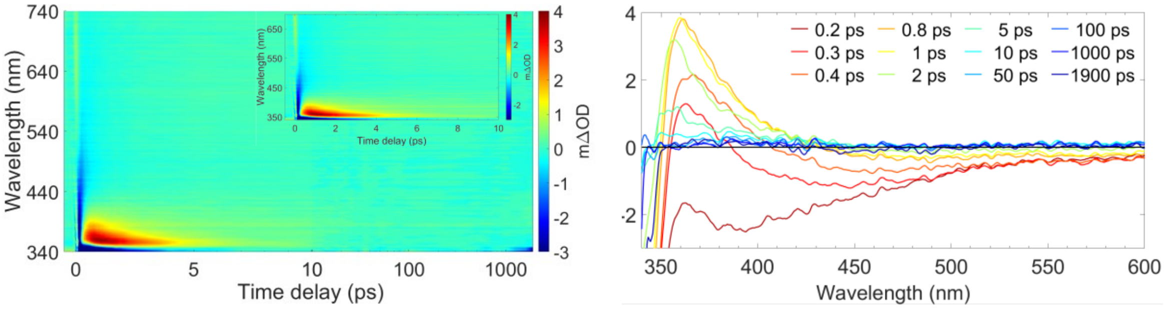

Fig. 5: TEAS spectrum of the undiluted commercial forms of Noriguard nc and Detoxophane nc, combined in a 1:1 ratio by volume, photoexcited at 330 nm, with 100 µm path length. (Left) False colour heat map showing all transient absorption spectra (TAS) of the 1:1 mixture. The time delay axis is linear up to 10 ps and logarithmic thereafter. Inset is a zoom-in of the TAS up to 10 ps, presented on a linear scale. (Right) TAS at selected pump-probe time delays: these plots are attained by taking vertical slices through the false colour heat maps at the given time delay and are presented on the same scale as the heat map.

nc was added in equal parts to Noriguard nc. These results are very similar to those presented in Fig. 4, with very few changes observed despite the presence of Detoxophane nc in the same volume. This was also the case for a 2:1 ratio of Detoxophane nc to Noriguard nc, hence the results are not shown in this report. To see the typical TEAS spectral signals from SM and Detoxophane nc that have previously been identified (6, 17, 18) the proportion of Detoxophane nc was significantly increased to 10:1. The result of this increase is shown in Fig. 6. In this instance, the spectral features align much more closely with SM and garden cress sprout extract, although the data is noisy due to low signal. The indication

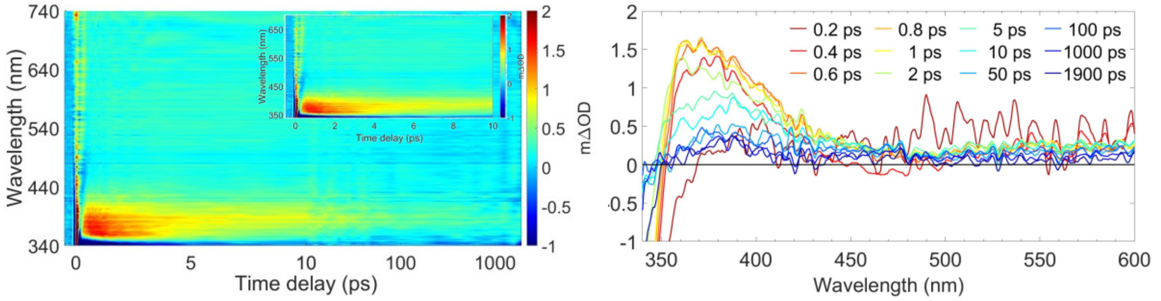

Fig. 6: TEAS spectra of the commercial forms of Noriguard nc and Detoxophane nc, in 1:10 ratio by volume, photoexcited at 330 nm using 100 µm spacers. (Left) False colour heat map showing all transient absorption spectra (TAS) of the 1:10 mixture. The time delay axis is linear up to 10 ps and logarithmic thereafter. Inset is a zoom-in of the TAS up to 10 ps, presented on a linear scale. (Right) TAS at selected pump-probe time delays: these plots are attained by taking vertical slices through the false colour heat maps at the given time delay and are presented on the same scale as the heat map.

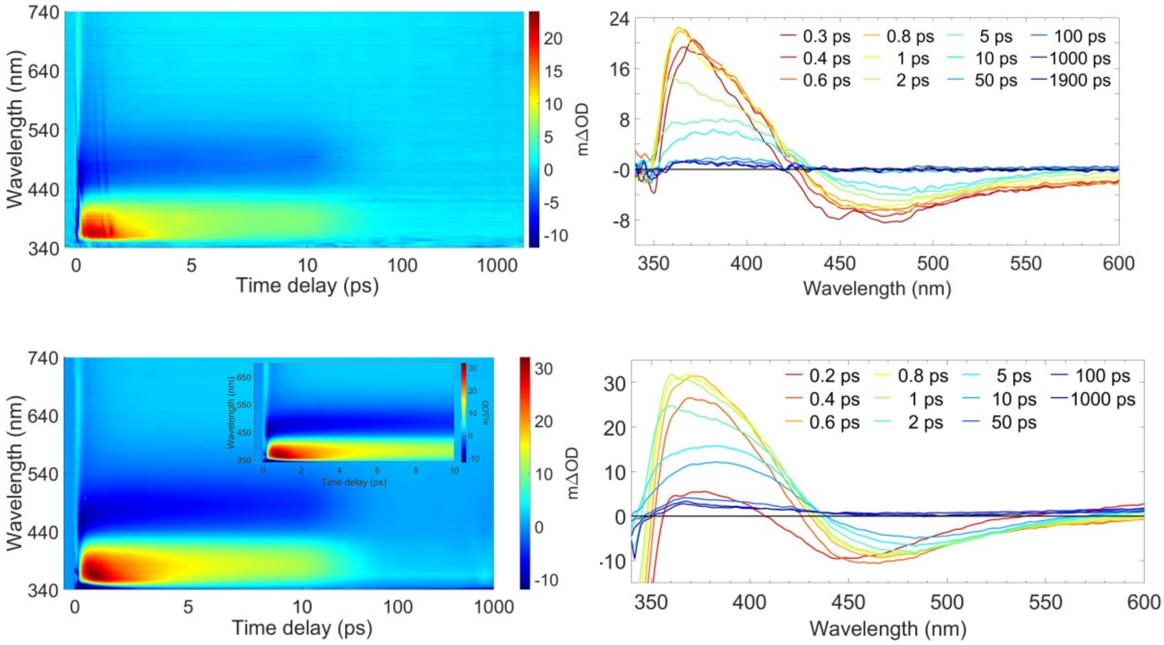

Fig. 7: Top row: TEAS spectrum of garden cress sprout extract added to Noriguard nc, photoexcited at 330 nm, with a 950 µm path length used to improve signal, as opposed to 100 µm in earlier measurements. (Left) False colour heat map showing all transient absorption spectra (TAS) of the mixture. The time delay axis is linear up to 10 ps and logarithmic thereafter. (Right) TAS at selected pump-probe time delays. Bottom row: TEAS spectra of the dried garden cress sprout extract and Nori red algae extract, combined in a 1-to-1 ratio by mass in DI water solvent, photoexcited at 330 nm, with 950 µm path length. It is noted that, due to experimental constraints, the maximum time delay for these measurements is 1 ns. (Left) False colour heat map showing all transient absorption spectra (TAS) of the 1:1 mixture. The time delay axis is linear up to 10 ps and logarithmic thereafter. Inset is a zoom-in of the TAS up to 10 ps, presented on a linear scale. (Right) TAS at selected pump-probe time delays.

from these results is that there is little interaction between the active ingredients when combined directly, one seems to solely dominate the other.

To investigate further, the effect of the garden cress sprout extract from Detoxophane nc being free from the encapsulated environment was investigated using TEAS. The extract was combined with the commercial form of Noriguard nc in the first instance. 0.3% w/w of garden cress sprout extract was added to mimic 1 to 1 ratio of active ingredient in Detoxophane nc to Noriguard nc, without effect of encapsulation of garden cress sprout extract. In addition, a 1:1 mixture by mass of the two dried extracts together was measured. This was to assess whether the presence of additional ingredients in Noriguard nc would have any impact on the synergetic effect with Detoxophane nc, compared to the Nori extract alone. The results of these measurements are given in Fig. 7.

Changing to the dried garden cress sprout extract free from encapsulation highlights SM signals in the TEAS data, which have been identified in previous work at the University of Warwick. (15, 19) Thus far, it had not been possible to observe these signals. In addition, the garden cress sprout extract has been shown to contain a high concentration of SM (around 10%). (6) This high concentration is evident from the data, as the spectral signals of MAAs as shown in Fig. 4, for example, now cannot be easily identified.

Discussion

From the irradiation results in Fig. 3, it can be concluded that a combination of these two ingredients could offer broad-spectrum UV boosting potential. The percentage decrease in absorption from the commercial forms alone can be considered very low and hence a good initial indication of the UV boosting properties of both ingredients. Further to this, the combination of the two ingredients seems to display evidence of the synergetic effects that we hoped to see. This could be due to spectral overlap of the two ingredients (both absorbing UV radiation in the same region), therefore the incident UV light can be "spread" across the two ingredients and increase their overall photostability. Equally, some energy transfer could occur between the UV active ingredients, making photodegradation less likely. The higher photostability of the commercial forms compared to the dried extracts in water, is most likely due to the presence of additional ingredients in the commercial forms that can absorb and/or scatter UV radiation. Moreover, the garden cress sprout extract in Detoxophane nc is encapsulated, thus preventing the UV radiation from reaching the extract in the first instance. However, the 10% reduction in UV absorption demonstrated by the two dried extracts in Fig. 4 still constitutes very good UV photostability. It is also an improvement on the 18% reduction in absorbance found by Abiola *et al.* for the garden cress sprout extract alone (6), suggesting that the addition of the second extract is having a shielding effect. The low SPF reported for Detoxophane nc, despite being a UVB absorber, can possibly be attributed to the encapsulation of the garden cress sprout extract, thus reducing the active concentration of UVB filtering compounds. However, this small value shows limited possibility for UV boosting, with potential to increase this further by creating a more potent cress extract.

The commencement of the ultrafast spectroscopy studies focussed upon Noriguard nc alone initially, to build upon previous studies.The spectral features of Noriguard nc alone are important to establish so that the effect of additional components can be characterised. Overall, the similarity between the spectral features of previously studied MAAs and Helioguard™ 365 is a positive outcome, as it suggests that combining the Nori extract with other products, such as Detoxophane nc, would not adversely impact the exemplary UV boosting properties of MAAs. The inability to identify spectral signals from SM is likely due to the encapsulation of the garden cress sprout extract, which will prevent the two plant extracts from interacting with one another on a molecular level. The encapsulation will also decrease the amount of light absorbed by the chromophores in this extract, thus reducing the signal. There are also differences between the concentrations of the UV absorbing components of Detoxophane nc and Noriguard nc, which is also a contributing factor. The results using the garden cress sprout extract alone show that the concentration of SM in the extract is higher that the concentration of MAAs in the Nori extract, however it was not possible to quantify the differences during this study.

An interesting result was uncovered when the results from Fig. 6 (ten parts Detoxophane nc to one part Noriguard nc) and Fig. 7 (using garden cress sprout extract) were compared. There is a stimulated emission feature present for the dried extract in the wavelength region of *ca.* 450 nm, which is not present in measurements of Detoxophane nc. This demonstrates that Detoxophane nc shows the traits of SM in a non-polar solvent, whereas the dried extract is indicative of the features of this molecule in a polar solvent. It is therefore apparent from the ultrafast spectroscopy measurements that the encapsulation of the extract in the commercial form is shielding SM from the effects of the polar water. As such, a synergetic effect will perhaps be enhanced by the extract not being encapsulated. It is difficult to conclude from TEAS measurements alone, as it has not been possible to observe both ingredients at the same time, due to concentration differences between the chromophores.

Conclusion

This collaboration between Mibelle Group Biochemistry and the University of Warwick aimed to determine whether a synergetic effect existed between two ingredients: Detoxophane nc, containing sinapoyl malate, and Noriguard nc, containing mycosporine-like amino acids. **The combination of active ingredients from both the land and the sea is highly innovative and unique.** The irradiation results show very promising evidence of a synergy between the two aspects of nature, as there was an excellent improvement in photostability when the two were combined. Together, they also have the potential to create a broad-spectrum UV boosting effect. However, more work is needed to investigate the true efficacy of the dried garden cress sprout extract. TEAS (alongside supplementary techniques) can be a valuable tool for cosmetic scientists and used successfully alongside conventional efficacy testing used in industry (e.g. *in vitro* SPF testing). In this case, it was difficult to observe the reasons for the synergies, as the signals from the more concentrated ingredient masked the other. This was the main limitation of the study. Future work will seek to overcome this challenges, perhaps progressing to the effect that these nature-based ingredients can have in a formulation environment.

The scope of this study has also been widened to investigate whether the nature-based ingredients can be used as a shield to protect traditional UV filters, which are known to be photolabile. Preventing the degradation of traditional UV filters is a crucial challenge, and the techniques used in this study are valuable investigative tools towards achieving this goal. Furthermore, additional UV shielding from compounds found in nature opens the possibility for decreasing the quantity of UV filters incorporated into a formulation. Encouraging results could widen the applications of these nature-based ingredients in the cosmetic science field.

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