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Transformation of 2,4,6-trimethylphenol and furfuryl alcohol, photosensitised by Aldrich humic acids subject to different filtration procedures

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URL: http://chimica.campusnet.unito.it/do/docenti.pl/Show?_id=dvione.

Abstract

Suspended particles in a system made up of Aldrich humic acids (HA) in water account for about 13% of the total HA mass, 10-11% of the organic carbon and 9-11% of radiation extinction in the UVA region. Extinction would be made up of radiation scattering (less than one third) and absorption (over two thirds). The contribution of particles to the degradation rates of trimethylphenol and furfuryl alcohol (FFA) (probes of triplet states and ¹O₂, respectively) was lower than 10% and possibly negligible. The results indicate that triplet states and ¹O₂ occurring in the solution bulk are mostly produced by the dissolved HA fraction. Experimental data would not exclude production of ¹O₂ in particle hydrophobic cores, unavailable for reaction with FFA. However, the limited to negligible particle fluorescence places an upper limit to particle core photoactivity.

Keywords: photosensitisers; humic acid triplet states; singlet oxygen; spectral slope; radiation absorption.

Introduction

Humic substances (HS) are important photosensitisers in soil and environmental systems, due to their ability to produce reactive species under irradiation (Canonica, 2006; Guerard et al., 2009). Some HS chromophores absorb sunlight and reach excited singlet states which can undergo fluorescence decay that allows characterisation (Fu et al., 2002). An alternative process is inter-system crossing to triplet states ($^3\text{HS}^*$). $^3\text{HS}^*$ are chemically reactive (by electron or hydrogen abstraction or energy transfer), and account for most of the sensitising HS activity (Werner et al., 2005; Canonica, 2007; Chen et al., 2009). A major example of energy transfer is reaction with Q to yield $^1\text{O}_2$, which can be involved into the transformation of easily oxidised compounds (Aguer et al., 1999). Irradiated HS can produce H_2O_2 (Al Housari et al., 2010; Page et al., 2011), partially because some triplet states are able to oxidise H_2O and possibly water (Kitamura et al., 1997; Sur et al., 2011). Moreover, the reduction of $^3\text{HS}^*$ to HS^- upon electron transfer (or to HS^\cdot upon hydrogen transfer) may be followed by re-oxidation which gives $\text{HO}_2^\cdot/\text{O}_2^{\cdot-}$, which produce H_2O_2 by disproportionation. Hydrogen peroxide can then produce $^1\text{O}_2$ via direct photolysis or Fenton and photo-Fenton processes (Mostofa and Sakuma, 2009; Vermilyea and Voelker, 2009).

Recent studies have been focused on the photogeneration of reactive species by HS and particularly humic acids (HA). By use of furfural or furfuryl alcohol (FFA) as $^1\text{O}_2$ probes, it has been shown that low-molecular weight HA fractions most effectively produce $^1\text{O}_2$ under irradiation (Cavani et al., 2009; Trubetskoj et al., 2009). However, recent results suggest a considerable amount of $^1\text{O}_2$ may be produced in hydrophobic sites of larger HA particles, which would not be readily available for reaction with hydrophilic probes (Appiani and Hill, 2011). Therefore, the issue of photoactivity of smaller vs. larger HA fractions and particles is still a matter of debate. To our knowledge, most recent studies of HA fractions photoactivity have been focused on $^1\text{O}_2$ photoproduction rather than triplet-state generation. TMP (2,4,6-trimethylphenol) is a very suitable molecule to probe $^3\text{HA}^*$, because it undergoes sufficiently selective reaction (Djalle et al., 2007). In the present study we used TMP and FFA as probes for $^3\text{HA}^*$ and $^1\text{O}_2$, respectively, photoproduced in aqueous solution by Aldrich HA. The latter underwent different filtration procedures (at 5, 0.45 and 0.10 μm pore-size diameter) to gradually yield suspended particles. The different HA fractions were characterised by dynamic light scattering and nephelometry, to assess the presence of suspended particles, by total organic carbon and various optical properties (absorbance and excitation-emission matrix fluorescence spectra). The results provide insight

membranes of 100 mg L⁻¹ HA yielded 12.8±0.1 mg L⁻¹ of retained material on the filter. Therefore, a detectable fraction (~13%) of HA in water would be “suspended”. Note that further filtration at 0.10 µm was not expected to remove a significant fraction of the HA mass (*vide infra*). The amount of organic matter removed by different filtration procedures was determined from the TOC content of various 10 mg L⁻¹ HA fractions (Table 1). Statistically insignificant differences of TOC values between original and 5 µm and between 0.45 and 0.10 µm fractions suggest that 0.45 µm filtration would remove most of the suspended organic material (very probably, most of the total material as well). Moreover, comparison between the TOC content of original HA and of HA filtered at 0.45 and 0.10 µm suggests that suspended material accounts for (10.6±3.2)% of organic carbon in the original sample.

The above framework was confirmed by dynamic laser light scattering (DLS). Systems containing 10 mg L⁻¹ HA were analysed as prepared and after filtration on 5, 0.45 and 0.10 µm pore-diameter filters. Significant scattering was only recorded in the presence of original and 5 µm-filtered solutions, which allowed an estimate of the hydrodynamic particle radii (see Figure SM1-A). The 5-µm filtration decreased the average hydrodynamic radius from 130±16 to 92±13 nm. The autocorrelation functions after 0.45 and 0.10 µm filtration, together with the signal of Milli-Q water, were recorded showing just minor differences (see Figure SM1-B). It was not possible to accurately assess the size distribution of the remaining particles after filtration at 0.45 and 0.10 µm, thus one can conclude that particles were still detectable but their amount was almost negligible. Filtration results as checked by DLS indicate that filters removed smaller particles than their nominal pore-size diameter would suggest. Possible explanations are that: (i) filters have a large pore-size distribution, the nominal one just being the most likely; (ii) HA particles may not be spherical; and/or (iii) groups of small, soft particles might easily clog larger filter pores.

As far as nephelometry is concerned, a linear calibration function with formazine suspension was obtained in the whole instrumental range (0-700 NTU, see Figure SM2). A detection limit (LOD) of 0.6 NTU (three times the standard deviation of the intercept) was also obtained under the adopted conditions. The linearity of the instrumental response up to 700 NTU means that the upper turbidity value was safely far from 100% radiation scattering, under which conditions one would expect saturative behaviour. Hereafter, 700 NTU will be assumed as a safe lower limit for 100% scattering. Turbidity values of 10 mg L⁻¹ HA, original and filtered at 5, 0.45 and 0.10 µm were respectively 3.8 NTU, 2.5 NTU, < LOD, and < LOD. The two latter figures confirm removal of most of the particles by filtration at 0.45 or 0.10 µm. An upper limit of $[100 \cdot (1 - 0.056)^{-1} \cdot (3.8 \text{ NTU}) \cdot (700 \text{ NTU})^{-1}] \% = 0.57\%$ was obtained for radiation scattering by

particles contained in original HA, after correcting for the fact that such a system would absorb about 5.6% of radiation emitted by the nephelometer lamp (see Figure SM3 and related discussion). As a comparison, extinction by particles (absorption + scattering) of nephelometer lamp radiation would be around 1.7% (see SM). Therefore, less than one third of the extinction would be accounted for by scattering and over two thirds by absorption. This is probably a large overestimate for scattering, because 700 NTU would be a very low limit for 100% scattering. Therefore, particles are expected to significantly absorb radiation.

The spectra of HA subject to different filtration procedures have small but observable differences (Figure 1), which are accounted for by particle radiation extinction (absorption + scattering, the latter expectedly minor). Under UVA irradiation conditions, the photon flux extinguished by the different HA fractions is $P_a = \int_{\lambda} p^{\circ}(\lambda)(1 - 10^{-A_1(\lambda)b})d\lambda$. Here $p^{\circ}(\lambda)$ is the spectral photon flux density of the UVA lamp, reported in Figure 1 together with $A_1(\lambda)$ of HA, and $b = 1.6$ cm is the optical path length of the irradiated systems. The P_a values reported in Table 1 show variations of around 9-11% between original HA and the fractions filtered at 0.45 and 0.10 μm . Such a difference is not far from the ~13% mass fraction of suspended material or the ~10% fraction of suspended organic carbon. Considering that radiation absorption would prevail over scattering (under the hypothesis that the absorption/scattering ratio determined by nephelometry is also valid under UVA), one can conclude that particles absorb radiation per unit carbon mass to a comparable extent as dissolved species.

The values of $A_{254} \text{ TOC}^{-1}$ (Table 1), where A_{254} is the absorbance at 254 nm with $b = 1$ cm, are above $6 \cdot 10^{-2} \text{ L (mg C)}^{-1} \text{ cm}^{-1}$ as expected for HA (Oliveira et al., 2006). The spectral slope S was determined by fitting the $A_1(\lambda)$ data of Figure 1 (300-540 nm interval) with the exponential equation $A_1(\lambda) = A e^{-S\lambda}$ (Bracchini et al., 2005). When referred to actual absorption of radiation, and not to scattering, the value of S is inversely proportional to the molecular weight of organic matter (Peuravuori and Pihlaja, 1997). The overall increase of S upon filtration (from $10.6 \pm 0.1 \mu\text{m}^{-1}$ for original HA to $11.1 \pm 0.1 \mu\text{m}^{-1}$ for the 0.1 μm fraction, Table 1) would reflect the elimination of the larger components. Coherently, the S value of the difference between the spectra of original and 0.1- μm filtered HA, which would be referred to suspended particles, is $7.5 \pm 0.1 \mu\text{m}^{-1}$. The overall coherence of these findings constitutes additional evidence that HA particles are able to significantly absorb radiation.

The fluorescence excitation-emission matrices (EEM) of 10 mg L^{-1} original and filtered HA were recorded and elaborated as contour plots (see Figure SM4). EEMs are dominated by two main elements, linear features and fluorescence peaks. The linear features are the first and second

harmonic of the Rayleigh-Tyndall scattering (where emission equals or doubles the excitation wavelength), as well as the Raman scattering of water (small signal with red-shifted emission wavelengths compared to excitation ones) (Baker, 2002). Fluorescence peaks are located at Ex_{max}/Em_{max} 250-275 nm/400-480 nm and Ex_{max}/Em_{max} 340-360 nm/420-480 nm. Both peaks (A and C according to Coble, 1996) are related to the presence of humic-like substances, while the absence of tryptophan and tyrosine-like peaks allows exclusion of protein-like material. The most evident consequence of filtration is the decrease of the Rayleigh-Tyndall scattering upon gradual elimination of particles.

Figure 2A reports fluorescence spectra at fixed excitation wavelength (260 nm). The first harmonic signals (scattering, $Ex = Em = 260$ nm) decrease progressively with filtration, in general agreement with DLS and nephelometry data. The fluorescence peaks are only slightly modified by filtration, which suggests that they are mainly related to dissolved species. Figure 2B shows the zoom in the 350-450 nm range of the spectra obtained at $Ex = 260$ nm, and indicates that the “peak A” intensity (Coble, 1996) even increases with filtration. It is suggested either a quenching effect of particles on the fluorescence of dissolved species, or a competition for radiation absorption between particles and dissolved compounds. Such a competition would decrease radiation absorption by dissolved species and, hence, their fluorescence intensity. Anyway, fluorescence by HA particles is expected to be very low to nil, and its percentage contribution would be much lower than the ~10% radiation extinction by particles in the unfiltered HA system.

Sensitised transformation of TMP and FFA under irradiation.

Figure 3 shows the time trends of 0.3 mM TMP (3A) and 0.1 mM FFA (3B) upon UVA irradiation of 10 mg L^{-1} HA, original and filtered at 5, 0.45 and 0.10 μm . Negligible transformation of TMP and FFA was observed in the dark, or upon irradiation in Milli-Q water.

Very similar trends were observed with the different HA fractions, with practically overlapping curves. The corresponding initial transformation rates of TMP and FFA (R_{TMP} and R_{FFA} , see Table 1) are also very close, within experimental errors. Such observations are supported by the *t*-test at 95% confidence level, which indicates that differences between R_{TMP} and R_{FFA} are insignificant for the various HA fractions.

The transformation of TMP is mostly accounted for by $^3\text{HA}^*$, that of FFA by $^1\text{O}_2$ (Halladja et al., 2007). Note that $^1\text{O}_2$ would be mostly produced by reaction between $^3\text{HA}^*$ and O_2 (Aguer et al., 1999), thus both measurements give insight into the processes that are sensitised (directly or *via* $^1\text{O}_2$) by $^3\text{HA}^*$.

Discussion

Suspended particles in aqueous HA, which are already largely removed by filtration at 0.45 μm , account for approximately 10% of the total HA mass and organic carbon content. They give a contribution of about the same percentage to the $A_1(\lambda)$ spectrum between 300 and 540 nm, as indicated by P_a calculations. Nephelometric measures suggest that the majority of radiation extinction by particles would be accounted for by absorption, with an upper limit for scattering of about one third of total extinction (the actual importance of scattering is probably much lower).

The percentage contribution of suspended particles to the transformation of TMP and FFA would be considerably lower than 10%, and possibly negligible. In the case of FFA the present finding agrees with previous studies, showing that low molecular weight HA fractions give the most important contribution to FFA transformation (Cavani et al., 2009). The TMP data reported in this study extend such an observation to a $^3\text{HA}^*$ probe. Therefore, most of TMP and FFA transformation would be accounted for by the dissolved HA fraction.

A comparison between optical properties of suspended particles and dissolved compounds in HA can be carried out, by considering the values of P_a and TOC. The properties of dissolved species can be reasonably derived by considering the 0.10 μm fraction, which had $P_a = 1.04 \cdot 10^{-6}$ Einstein $\text{L}^{-1} \text{s}^{-1}$, $\text{TOC} = 3.90 \pm 0.05$ mg C L^{-1} , and radiation absorption per unit organic carbon, $P_a \text{ TOC}^{-1} = (2.67 \pm 0.03) \cdot 10^{-7}$ Einstein (mg C) $^{-1} \text{s}^{-1}$. The corresponding properties of suspended particles could be obtained by difference (total – dissolved), between the P_a and TOC values of original HA and those of the 0.10 μm fraction. For particles, $P_a = 1.3 \cdot 10^{-7}$ Einstein $\text{L}^{-1} \text{s}^{-1}$, $\text{TOC} = 0.46 \pm 0.13$ mg C L^{-1} , and $P_a \text{ TOC}^{-1} = (2.83 \pm 0.80) \cdot 10^{-7}$ Einstein (mg C) $^{-1} \text{s}^{-1}$. Note the very similar values of $P_a \text{ TOC}^{-1}$ obtained for both dissolved species and particles. Because radiation absorption by particles would prevail over scattering, it is suggested that dissolved species and particles have very similar chromophores. Such a similarity, which would be correlated with $^1\text{O}_2$ photoproduction (Vione et al., 2010) is hardly reflected in the degradation rates of TMP and FFA that are negligible for particles.

A possible explanation of this apparent contradiction would take into account the microheterogeneity of $^1\text{O}_2$ distribution within HA. Very high steady-state [$^1\text{O}_2$] has been found in hydrophobic sites, as indicated by higher transformation rates for $^1\text{O}_2$ hydrophobic probes compared to FFA (Latch and McNeill, 2006; Grandbois et al., 2008). Hydrophobic sites should also be common in suspended particles (Appiani and McNeill, 2011). TMP and most notably FFA are water-soluble molecules, which would mostly react with $^3\text{HA}^*$ and $^1\text{O}_2$ that occur in the solution

bulk. As shown here, bulk species are mostly produced by the dissolved HA fraction. Conversely, reaction between TMP or FFA and particle-produced $^3\text{HA}^*$ or $^1\text{O}_2$ should involve the probe molecules that occur near the particle surface, and the surface-produced reactive species. Reaction would thus exclude probe molecules in the bulk and reactive transients in the particle core, which are likely to make up the vast majority of probes and particle-produced transients.

Note that higher transformation rates of hydrophobic $^1\text{O}_2$ probes compared to FFA (Appiani and McNeill, 2011) would not imply that particles produce much more $^1\text{O}_2$ per unit organic carbon than dissolved species. Considering the correlation between absorption properties and $^1\text{O}_2$ photogeneration rates (Vione et al., 2010), a hypothesis of highly photoactive particles would be poorly consistent with our $P_a \text{ TOC}^{-1}$ values, which are similar for both dissolved species and particles. High particle photoactivity would also be surprising, given the results of our measurements that suggest low to negligible particle fluorescence, and even a slight quenching by particles on the fluorescence of dissolved species. A recent survey study has found a strong link between fluorescence intensity and $^1\text{O}_2$ photoproducton by different humic substances (Coelho et al., 2011). This result may look surprising (fluorescence and triplet formation are competitive processes for a single molecule), but it is easily explained for a class of compounds such as HA. Fluorescence and triplet formation are in fact both favoured, obviously to a different extent for different single compounds, when other processes such as internal conversion are relatively slow.

Our results exclude that $^1\text{O}_2$ reacting with FFA in solution may be the fraction that escapes out of the particle hydrophobic core. Insignificant difference in FFA degradation was observed upon particle elimination, which implies that most $^1\text{O}_2$ occurring in solution was produced by dissolved HA. In the solution bulk, the main quenching process for $^1\text{O}_2$ is collision with water to give O_2 (rate constant $2.5 \cdot 10^5 \text{ s}^{-1}$) (Rodgers and Snowden, 1982). Reaction with water would not be operational in HA hydrophobic sites, where the lifetime of $^1\text{O}_2$ could be much longer than in solution. Therefore, one could observe faster degradation of $^1\text{O}_2$ hydrophobic probes in particles than of hydrophilic ones in solution, even with lower $^1\text{O}_2$ formation rates in particles.

Conclusions

This work shows that suspended particles in an Aldrich HA – water system account for about 13% of the total HA mass (the remainder being accounted for by dissolved species), for around 10-11% of the organic carbon, and for 9-11% of the measured UVA radiation absorption (actual absorption is expected to prevail over scattering). The contribution of particles to the degradation rates of TMP

and FFA (probes of $^3\text{HA}^*$ and $^1\text{O}_2$, respectively) was lower than 10% and possibly negligible. The water-soluble probes TMP and FFA would mostly react in the solution bulk, and our results indicate that bulk-available $^3\text{HA}^*$ and $^1\text{O}_2$ were mostly produced by the dissolved HA fraction. It can be excluded that bulk reactivity is dominated by reactive species that are produced by particles and migrate to the solution.

The present data would not exclude that particles may have hydrophobic cores where $^1\text{O}_2$ could reach high steady-state concentration, as suggested by recent findings (Appiani and McNeill, 2011), but they give suggestions on how these sites might behave. First of all, hydrophobic cores could not be a significant source of bulk $^1\text{O}_2$. It is also unlikely that they afford an unusually high formation rate of $^1\text{O}_2$, considering that the particle optical properties are not much different from those of the dissolved HA fraction, and that absorption properties are correlated with $^1\text{O}_2$ photogeneration. Moreover, considering the very low to nil fluorescence intensity that would also be correlated with $^1\text{O}_2$ photoproduction, particles are expected to be even less photoactive than dissolved species.

The most likely explanation of particle core photoactivity is that hydrophobic sites do not contain water, which is a very effective $^1\text{O}_2$ quencher (Rodgers and Snowden, 1982). Therefore, higher lifetime of $^1\text{O}_2$ in hydrophobic environments could lead to more effective reactions.

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Table 1. Principal parameters measured on 10 mg L⁻¹ HA (original and filtered at 5, 0.45 and 0.1 μm). Ein = Einstein. **: impossible to determine. LOD: Limit Of Detection.

	Original	Filtered 5 μm	Filtered 0.45 μm	Filtered 0.10 μm
P_a, Ein L⁻¹ s⁻¹	1.17·10 ⁻⁶	1.12·10 ⁻⁶	1.07·10 ⁻⁶	1.04·10 ⁻⁶
TOC, (mg C) L⁻¹	4.36±0.08	4.33±0.06	3.90±0.06	3.90±0.05
P_a / TOC, Ein s⁻¹ (mg C)⁻¹	(2.68±0.05)·10 ⁻⁷	(2.58±0.04)·10 ⁻⁷	(2.74±0.04)·10 ⁻⁷	(2.67±0.03)·10 ⁻⁷
A_{254nm}, cm⁻¹	0.294	0.289	0.285	0.264
A_{254nm} / TOC, L (mg C)⁻¹ cm⁻¹	(6.74±0.12)·10 ⁻²	(6.67±0.09)·10 ⁻²	(7.31±0.11)·10 ⁻²	(6.77±0.09)·10 ⁻²
S, μm⁻¹	10.6±0.1	10.9±0.1	11.4±0.1	11.1±0.1
Turbidity, NTU	3.8	2.5	<LOD	<LOD
Hydrodynamic radius, nm	130±16	92±13	**	**
R_{TMP}, M s⁻¹	(1.07±0.04)·10 ⁻⁸	(9.32±0.26)·10 ⁻⁹	(1.06±0.02)·10 ⁻⁸	(1.12±0.04)·10 ⁻⁸
R_{FFA}, M s⁻¹	(6.36±0.20)·10 ⁻¹⁰	(5.49±0.12)·10 ⁻¹⁰	(6.03±0.21)·10 ⁻¹⁰	(6.22±0.15)·10 ⁻¹⁰

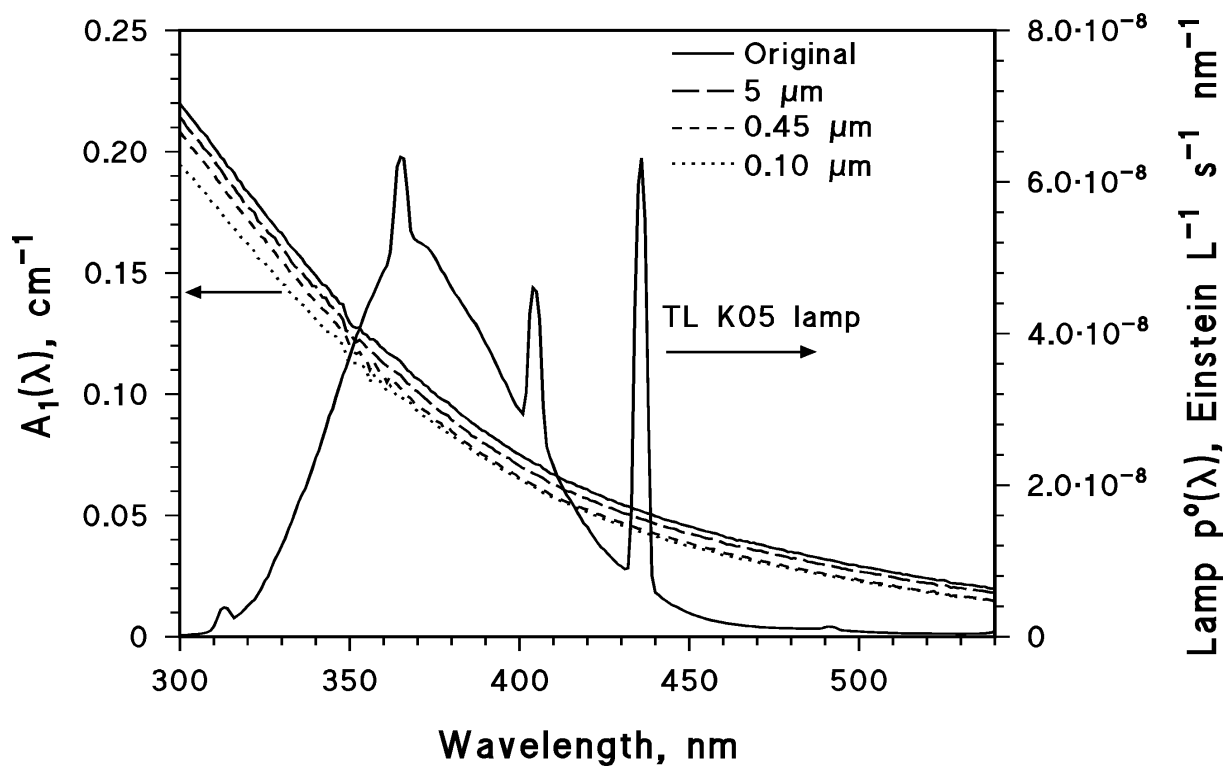


Figure 1. (Left Y-axis) Absorbance (optical path length $b = 1$ cm) of 10 mg L^{-1} HA, original and filtered at 5, 0.45 and $0.10 \mu\text{m}$. (Right Y-axis) Spectral photon flux density in the irradiated solutions.

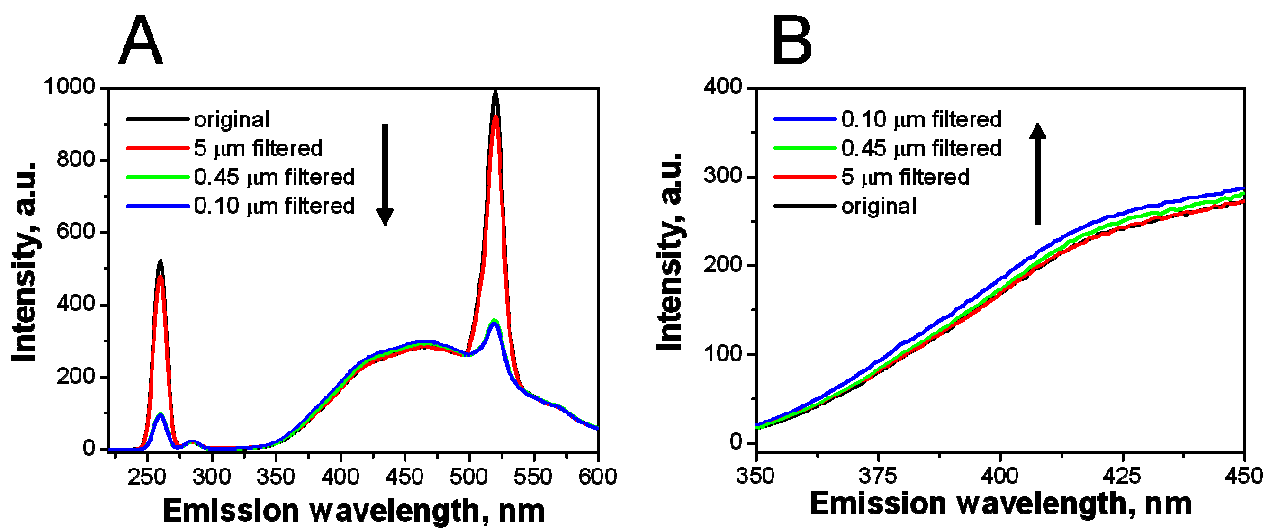


Figure 2. Fluorescence spectra at fixed 260 nm excitation wavelength of 10 mg L⁻¹ HA samples (original and filtered at 5, 0.45 and 0.1 μm): (A) full scan; (B) zoom in the 350-450 nm region.

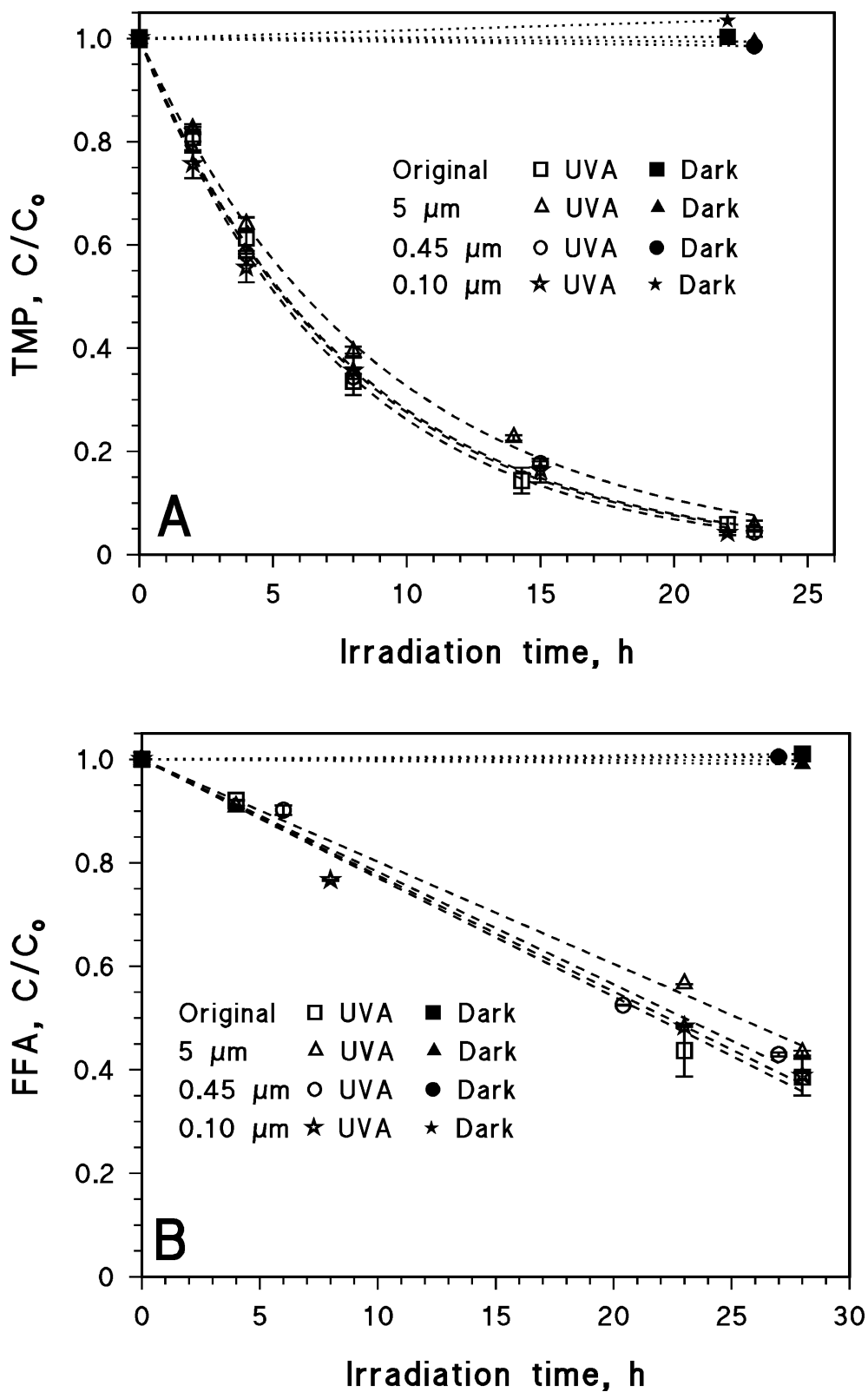


Figure 3. Time trends of (A) 0.3 mM TMP and (B) 0.1 mM FFA under UVA irradiation in the presence of 10 mg L⁻¹ HA samples (original and filtered at 5, 0.45 and 0.10 μm), both in the dark and under irradiation.