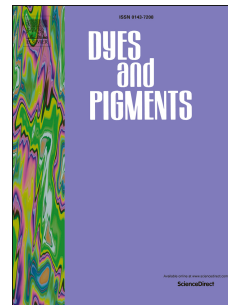# Accepted Manuscript

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A comprehensive spectral, photophysical and electrochemistry study of synthetic water-soluble acridones. A new class of pH and polarity sensitive Fluorescent Probes

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ABSTRACT

A comprehensive spectroscopic, photophysical and electrochemical investigation of *N*-alkylacridones, their newly synthesised chlorosulfonyl and water-soluble sulfonic acid derivatives has been undertaken in solution at room temperature. The study includes absorption and emission spectra together with quantitative measurements of the deactivation of the first excited singlet state, from which the rate constants for all the decay processes has been obtained. A comparison on the gradual change of the electronic spectral and photophysical properties with the degree of substitution is considered. In addition the electrochemical behaviour of the water-soluble acridones at different pH values was obtained by cyclic and differential pulse voltammetry. The oxidation mechanism of disulfonated acridone is found to occur in one consecutive and irreversible charge transfer pH-dependent reaction. The water-soluble acridones were found to display long lifetime values, dependent on the pH and solvent polarity. This opens a door for its use as selective probes for different cellular environment and targets.

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INTRODUCTION

Nowadays, there is a considerable interest in the development of fluorescence lifetime-based biological probes for assays in biophysics, clinical diagnostics, DNA analysis, and drug delivery. Unlike steady state fluorescence (based on emission variation of intensities), time-resolved fluorescence provides a signal (and value) that can be discriminate against many experimental artefacts resulting from steady-state measurements. Generally time-resolved fluorescence is independent of the probe concentration and volume, it remains unaffected by auto-fluorescence of biological samples or light scattering, and thus leads to more robust assays. Considering that fluorescent cellular (intrinsically) components typically have fluorescence lifetime shorter than 5 ns and that the more widely used (extrinsically) fluorophores such as fluorescein, rhodamine, coumarins or BODIPY´s[1-6] display lifetimes that are shorter than 10 ns, there is interest to develop fluorophores with long fluorescence lifetimes (with values of more than 10 ns), sensible to change in the environment and soluble in biological medium[7, 8].

Acridones, a subclass of acridines with a core structure consisting of 9(10*H*)-acridone, Scheme 1, are a group of compounds with applications in various fields, such as medicinal chemistry[9] namely as anti-viral[10, 11], anti-bacterial[12], anti-parasite, anti-fungi drugs[13], in single nucleotide polymorphism detection in DNA variation[14] and for the treatment of the malaria [15-17] and cancer[18-20].
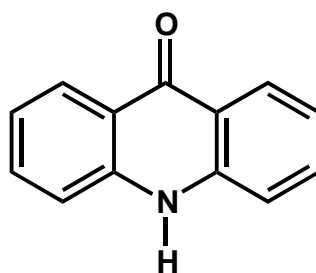
Scheme 1: Structure of Acridone

Acridone (AC) is also a well-known highly fluorescent fluorophore, with more than 50% of the quant loss made through the radiative channel [21, 22] and an emission (color) that is sensitive to its environment, which are both desirable attributes relevant to

designing media-sensitive chromophores for biolabeling purposes. Therefore, acridones and their derivatives have been used as chemosensors and fluorescent markers [23-29].

The spectroscopic characterization of 10-alkyl-9(10H)-acridone derivatives show the sensitivity of the absorption and emission maxima to the polarity of the solvent and the involvement of an electron transfer process in the fluorescence quenching [25]. Additionally, spectro-electrochemical experiments showed that the compounds oxidize irreversibly at low oxidation potential [30]. To date, the power conversion efficiency of solar cells using small molecules as donors, such as acridones, have exceeded 8%, making these materials ideal candidates for the solution processed Dye-Sensitized Solar Cells (DSSCs). [31, 32]

In our effort to obtain new probes presenting long-term stability for different applications including electrolytes for redox-flow batteries, sensing of oxygen, etc., we have made versatile acridones (and its alkyl derivatives) soluble in water with the introduction of the sulfonic group.[33, 34] In the present study, the spectral, photophysical and redox properties of the new water-soluble acridone derivatives were investigated in DMSO and water (at different pH values).

EXPERIMENTAL SECTION

Material and methods

Acridone (Aldrich), chlorosulphonic acid (Aldrich) and dimethyl sulfoxide (DMSO) of spectroscopic grade were used without further purification. Ultra-pure water at pH=5.4, was purified using a Direct Q3 Merck Millipor equipment. Ethyl Acetate was purified in reflux with potassium carbonate during 3 hours, distilled and then stored with molecular sieves (4 Å). When required, thin layer chromatography (TLC) silica plates 60 F250 (Merck or Fluka) were used. Absorption spectra were recorded on a Cary5000 UV-Vis-NIR spectrophotometer. Fluorescence spectra were recorded in a Horiba-Jobin-Yvon-Spex Fluorolog 3-2.2. spectrofluorimeter. The fluorescence quantum yields were determined using quinine bisulfate as standard ($\phi_F = 0.546$ in a 0.5 M H_2SO_4 aqueous solution).[35] The molar absorptivity constants (ε_{SS}, Table 1) were obtained by the adjustment of a slope on the plot of the absorption *vs.* concentration with correlation values above 0.99, obtained by preparing 4 solutions with concentrations ranging from $8x10^{-5}$ to $3x10^{-5}$ M.

The fluorescence decay times were obtained by the TCSPC (*Time-correlated Single Photon Counting*) technique with nanosecond resolution in an equipment described elsewhere[6, 36], and analysed using the method of modulating functions implemented by G. Striker[37]. Microwave assisted synthesis was carried out on a CEM Discover S-Class equipment with constant monitorization of temperature, pressure and potency.

NMR spectra were recorded on a Bruker Avance 400 spectrometer, operating at 400.13 MHz for ^{1}H NMR and 100.62 MHz for ^{13}C NMR. Chemical shifts (δ) are reported in ppm relatively to tetramethylsilane (TMS). High-resolution mass spectrometry analysis was carried out on a Bruker Microtof apparatus, equipped with selective ESI-TOF detector. Melting points were determined using a Melting Point Device Falc R132467 (open capillary method). Infrared spectra were recorded with an Agilent Technologies Carey 630 FTIR spectrometer with ATR.

General procedure for the synthesis of N-alkyl-9(10H)-Acridone derivatives.

The synthesis of the different *N*-alkyl acridone derivatives followed the synthetic procedure described by Wang *et. al.* [38]. To a mixture of acridone (0.100 g, 0.5 mmol), K_2CO_3 (0.0071 g, 0.18 mmol), Al_2O_3 (0.0533 g, 0.52 mmol) NaOH (0.0071 g, 0.18 mmol) and TBAB (0.0017 g, 0.005 mmol) the proper alkyl halide, iodomethane, iodopentane or iodooctane, (2 mL) was added on the 10 mL microwave tube. The mixture was heated under microwave irradiation during 15 min at 65 ºC with high speed stirring using a CEM (Discover SP) microwave oven. The reaction mixture was cooled at room temperature and the reaction product was purified by silica gel column chromatography using ethyl acetate and *n*-hexane (3:7) as eluent.

General procedure for the synthesis of N-alkyl-9(10H)-acridone-2,7-disulfonyl dichloride.

To the *N*-alkyl-9(10*H*)acridone (0.100 g) chlorosulfonic acid (2.5 mL, 0.38 mmol) was added and placed under inert atmosphere in a paraffine bath at 60 °C during 24 h at vigorous stirring. The solution is left to cool in ice and a saturated solution of $NaHCO_3$ (25 mL) was added. Then 20 mL of chloroform were added and the organic layer extracted. The organic layer was washed three times with water, dried with sodium sulfate

anhydrous and filtrated. The evaporation of chloroform under vacuum yield the *N*-alkyl-9(10*H*)-acridone-2,7-disulfonyl dichloride as yellow solids.

General procedure for the synthesisN-alkyl-9(10H)-acridone-2,7-disulfonic acids.

The chlorosulfonic derivatives were hydrolysed in water (50 mL) at 110 °C during 48 h. After cooling to room temperature the solvent was evaporated under vacuum yielding the corresponding disoulfonic acids.

Characterization

9(10H)-Acridone (from Sigma-Aldrich 99%)
(Yellow solid) Mp. 360-362 °C. ^{1}H NMR (400 MHz, DMSO-d6) δ(ppm) 11.73 (s, 1H), 8.23 (dd, *J* = 8.1, 1.3 Hz, 2H), 7.74 (dt, *J* = 8.4, 1.6 Hz, 2H), 7.55 (d, *J* = 8.2 Hz, 2H), 7.26 (dt, *J* = 8.0, 1.0 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ(ppm) 176.7, 140.8, 133.4, 125.9, 120.9, 120.4, 117.3. IR (ATR-FTIR) ν = 2939 (w, broadband), 1630 (m), 1591 (m), 1555 (m), 1528 (s), 1468 (s), 1338 (s), 1257 (m), 1156 (s), 1103 (m), 1021 (m), 934 (s), 812 (m), 747 (s), 669 (s).

9(10H)-Acridone-2,7-disulfonyl dichloride
(Pale Yellow solid)Yield: 28%. Mp. >300°C. ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm) 11.89 (s, 1H), 8.47 (d, *J* = 1.6 Hz, 2H), 7.92 (dd, *J* = 8.6, 1.9 Hz, 2H), 7.49 (d, *J* = 8.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ (ppm) 176.9, 141.3, 140.7, 131.2, 122.9, 119.3, 116.9. IR (ATR-FTIR) ν = 3253 (w), 3218 (w), 3183 (w), 3125 (w), 3020 (w), 1623 (s), 1599 (s) 1584 (m), 1515 (m), 1464 (m), 1375 (s), 1170 (s), 1130 (s), 1071 (s), 988 (m), 908 (m), 823 (s), 741 (m), 698 (s). MSHR *m/z* found [M+H]$^+$391.92170 molecular formula $C_{13}H_8Cl_2NO_5S_2$ requires 391.92150.

9(10H)-Acridone-2,7-disulfonic acid
(Yellow solid) Yield: 99%. Mp. >300 °C. ^{1}H NMR (400 MHz, DMSO-d6) δ(ppm) 11.86 (s, 1H), 8.47 (d, *J* = 1.1 Hz, 2H), 7.92 (dd, *J* = 8.6, 1.7 Hz, 2H), 7.49 (d, *J* = 8.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ(ppm) 176.9, 140.7, 131.2, 123.0, 119.3, 117.0. IR (ATR-FTIR) ν = 3258 (w, br), 1625 (w), 1585 (w), 1515 (w), 1473 (w), 1089 (s), 1032 (s), 823 (s) 709 (s). MSHR *m/z* found [M+H]$^+$355.98871 molecular formula $C_{13}H_{10}NO_7S_2$ requires 355.98932.

N-Methyl-9(10H)-Acridone
(Pale Yellow solid) Yield: 71.1%. Mp. 201-204 °C. ^{1}H NMR (400 MHz, CDCl$_3$) δ(ppm) 8.58 (dd, *J* = 8.0, 1.6 Hz, 2H), 7.75 (dt, *J* = 8.7, 1.7 Hz, 2H), 7.55 (d, *J* = 8.7 Hz, 2H), 7.31 (dt, *J* = 7.9, 0.8 Hz, 2H), 3.92 (s, 3H). ^{13}C NMR (101 MHz, CDCl$_3$) δ(ppm) 178.2, 142.6, 133.8, 127.8, 122.6, 121.3, 114.7, 33.7. IR (ATR-FTIR) ν = 2916 (w), 2847 (w), 1629 (m), 1589 (m), 1486 (m), 1459 (m), 1439 (m), 1366 (m), 1339 (m), 1287 (m), 1178 (m), 1127 (m), 1037 (m), 936 (m), 859 (m), 802 (m), 751 (s), 670 (s). MSHR *m/z* found [M+H]$^+$210.09180 molecular formula $C_{14}H_{12}NO$ requires 210.09130.

N-Methyl-9(10H)-Acridone-2,7-disulfonyl dichloride
(Pale Yellow solid) Yield: 30%. Mp. > 300 °C (degradation at 200 ℃). ^{1}H NMR (400 MHz, CDCl$_3$) δ(ppm) 9.19 (d, J = 2.5 Hz, 2H), 8.36 (dd, J = 9.2, 2.5 Hz, 2H), 7.80 (d, J = 9.3 Hz, 2H), 4.07 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d6) δ(ppm) 176.6, 142.2, 141.4, 131.5, 123.4, 120.5, 115.6, 34.1. IR (ATR-FTIR) ν = 3126 (w), 3075 (w), 2950 (w), 2853 (w), 1650 (m), 1592 (s), 1479 (m), 1371 (s), 1337 (m), 1279 (w), 1171 (s), 1147 (s), 1071 (s), 990 (w), 930 (w), 875 (w), 813 (s), 704 (s). MSHR *m/z* found [M+H]$^+$405.93560 molecular formula $C_{14}H_{10}Cl_2NO_5S_2$ requires 405.93720.

N-Methyl-9(10H)-Acridone-2,7-disulfonic acid

(Yellow solid) Yield: 99%. Mp. > 300 °C (degradation at 200 °C).^{1}H NMR (400 MHz, D$_2$O) δ(ppm) 8.65 (d, *J* = 2.2 Hz, 2H), 8.08 (dd, *J* = 9.2, 2.3 Hz, 2H), 7.77 (d, *J* = 9.2 Hz, 2H), 3.87 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d6) δ(ppm) 176.6, 142.1, 141.5, 131.5, 123.4, 120.5, 115.8, 34.1. IR (ATR-FTIR) ν = 3062 (w, broadband), 1617 (m), 1586 (m), 1551 (m), 1490 (m), 1282 (m), 1247 (m), 1197 (m), 1144 (m), 1085 (s), 1006 (s), 815 (s), 710 (s), 681 (s). MSHR *m/z* found [M+H]$^+$370.00413 molecular formula C$_{14}$H$_{12}$NO$_7$S$_2$ requires 370.00497.

N-Penthyl-9(10H)-Acridone

(Pale Yellow solid) Yield: 66.7%. Mp. 117-119 °C (yellow liquid).^{1}H NMR (400 MHz, CDCl$_3$) δ(ppm) 8.60 (dd, *J* = 8.0, 1.7 Hz, 2H), 7.74 (dt, *J* = 8.7, 1.7 Hz, 2H), 7.51 (d, *J* = 8.7 Hz, 1H), 7.31 (dt, 11.1, 3.9 Hz, 2H), 4.35 (t, 2H), 2.00-1.92 (m, 2H), 1.60-1.45 (bm, 4H), 1.00 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, CDCl3) δ(ppm) 178.0, 141.8, 133.9, 128.1, 122.5, 121.2, 114.5, 46.2, 29.1, 26.9, 22.5, 14.1. IR (ATR-FTIR) ν = 3075 (w), 2953 (w), 2925 (w), 2869 (w), 1631 (m), 1593 (s), 1484 (s), 1452 (s), 1373 (m), 1288 (m), 1262 (s), 1195 (m), 1172 (s), 1045 (m), 971 (m), 937 (m), 847 (m), 764 (s), 747 (s), 671 (s). MSHR *m/z* found [M+H]$^+$266.15380 molecular formula C$_{18}$H$_{20}$NO requires 266.15390.

N-Penthyl-9(10H)-Acridone-2,7-disulfonyl dichloride

(Pale Yellow solid) Yield: 69.9%. Mp. 188-190 °C.^{1}H NMR (400 MHz, CDCl$_3$) δ(ppm) 9.20 (d, *J* = 2.5 Hz, 2H), 8.36 (dd, *J* = 9.3, 2.5 Hz, 2H), 7.73 (d, *J* = 9.3 Hz, 2H), 4.44 (t, *J* = 8.5 Hz 2H), 2.05-1.97 (m, 2H), 1.57-1.48 (m, 4H), 1.02 (t, *J* = 7.2 Hz, 3H).^{13}C NMR (101 MHz, CDCl$_3$) δ(ppm) 175.4, 145.3, 138.4, 131.9, 129.0, 122.5, 116.9, 47.7, 28.8, 26.8, 22.4, 13.9. IR (ATR-FTIR) ν = 3072 (w), 2956 (w), 2928 (w), 2861 (w), 1657 (m), 1592 (m), 1478 (m), 1369 (m), 1276 (w), 1173 (m), 1145 (m), 1075 (m), 820 (m), 688 (m). MSHR *m/z* found [M+H]$^+$461.99970 molecular formula C$_{18}$H$_{18}$Cl$_2$NO$_5$S$_2$ requires 461.99980.

N-Penthyl-9(10H)-Acridone-2,7-disulfonic acid

(Yellow hygroscopic solid) Yield: 69.9%.^{1}H NMR (400 MHz, D$_2$O) δ(ppm) 8.67 (s, 2H), 8.05 (d, *J* = 9.0 Hz, 2H), 7.71 (d, *J* = 9.1 Hz, 2H), 4.27 (t, *J* = 8.5 Hz 2H), 1.73-1.67 (m, 2H), 1.39-1.24 (m, 4H), 0.80 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d6) δ(ppm) 176.5, 141.3, 131.7, 123.6, 120.5, 115.7, 30.6, 28.1, 26.6, 22.0, 13.9. IR (ATR-FTIR) ν = 3250 (w, broadband), 2956 (w), 2928 (w), 2865 (w), 1628 (m), 1584 (m), 1486 (m), 1172 (s), 1096 (s) 1027 (s), 983 (s), 811 (s), 710 (s). MSHR *m/z* found [M+H]$^+$426.066150 molecular formula C$_{18}$H$_{20}$NO$_7$S$_2$ requires 426.067570.

N-Octyl-9(10H)-Acridone

(Pale Yellow solid) Yield: 40%. Mp. 106-108 °C (yellow liquid).^{1}H NMR (400 MHz, CDCl$_3$) δ(ppm) 8.58 (dd, *J* = 8.0, 1.7 Hz, 2H), 7.73 (dt, *J* = 8.7, 1.7 Hz, 2H), 7.49 (d, *J* = 8.7 Hz, 2H), 7.29 (t, 2H), 4.33 (t, 2H), 1.97-1.89 (m, 2H), 1.61 – 1.50 (m, 2H), 1.50 – 1.40 (m, 2H), 1.38-1.29 (m, 4H), 0.91 (t, *J* = 6.9 Hz, 3H).^{13}C NMR (101 MHz, CDCl$_3$) δ (ppm) 178.0, 141.8, 133.9, 128.0, 122.5, 121.2, 114.5, 46.2, 31.8, 29.3 (d), 27.2, 26.9, 22.6, 14.1. IR (ATR-FTIR) ν = 2955 (w), 2912 (w), 2851 (w), 1630 (m), 1596 (m), 1493 (m), 1462 (m), 1378 (m), 1290 (m), 1266 (m), 1180 (m), 1044 (m), 938 (m), 747 (s), 669 (s). MSHR *m/z* found [M+H]$^+$308.20070 molecular formula C$_{21}$H$_{26}$NO requires 308.20090.

Voltammetric parameters and electrochemical cells

The 0.1 M ionic strength electrolyte solutions [39] at pH 0.0 H_2SO_4, pH 6.9 phosphate buffer and pH 13.0 NaOH/KCl were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity ≤ 10 μS/cm). Solutions of different concentrations were obtained by dilution of the appropriate volume in the supporting electrolyte. Nitrogen-saturated solutions were obtained by bubbling high purity N_2 for a minimum of 10 min in the solution and continuous flow of pure gas over the solution during the voltammetric experiments. All experiments were done at room temperature, T=298 K (25 °C). Microvolumes were measured using EP-10 and EP-100 Plus Motorized Microliter Pipettes (Rainin Instrument Co. Inc., Woburn, USA). The pH measurements were carried out with a Crison micropH 2001 pH-meter with an Ingold combined glass electrode. Voltammetric experiments were carried out using an Autolab potentiostat with GPES 4.9 software (Eco-Chemie, Utrecht, The Netherlands). The measurements were carried out in a solution volume of 3 mL, using a three-electrode system one-compartment electrochemical cell of capacity 2 mL.

A glassy carbon electrode (GCE, d = 1.5 mm) was the working electrode, a Pt wire the counter electrode and an Ag/AgCl (3 M KCl) reference electrode (Bio-Logic SAS, France). The experimental conditions were as follows: for cyclic voltammetry (CV), scan rate 100 mV/s; for differential pulse (DP), voltammetry pulse amplitude 50 mV, pulse width 70 ms and scan rate 5 mV/s. The glassy carbon electrode (GCE) was polished using a diamond spray (particle size 3 μm) (Kemet, UK) before each electrochemical experiment. After polishing, it was rinsed thoroughly with Milli-Q water. Following this mechanical treatment, the GCE was placed in a buffer supporting electrolyte, and differential pulse voltammograms were recorded until a steady-state baseline voltammogram was obtained. This procedure ensured very reproducible experimental results. Differential pulse voltammograms presented were background subtracted and baseline corrected using the moving average application with a step window of 5 mV included in the GPES v4.9 software.

This mathematical treatment improves the visualization and identification of peaks over the baseline without introducing any artefact, although the peak intensity is, in some

cases, reduced (10%) relative to that of the untreated curve. Nevertheless, this mathematical treatment of the original voltammograms was used in the presentation of all experimental voltammograms for an improved and clearer identification of the peaks. The values for peak current presented in all plots were determined from the original untreated voltammograms after subtraction of the baseline.

RESULTS AND DISCUSSION

Synthesis

N-alkyl-9(10*H*)-acridones were obtained adapting the method described by Wang *et al.*[38] for the synthesis of these compounds using a domestic microwave oven. The alkyl derivatives were obtained from a mixture of AC, Al_2O_3, NaOH, a catalytic amount of tetrabutylammonium (TBAB) and the respective alkyl halide: CH_3I, $nC_5H_{11}I$ or $nC_8H_{17}I$ in excess to act as solvent and reactant. Using a microwave reactor for synthesis with continuous monitoring of power, temperature and pressure it was possible obtain the desired products after 15 min at 65 ºC with initial power of 100 W, avoiding the use of solvent and in short reaction times when compared with conventional methodologies[40-42]. After purification by column chromatography the *N*-alkyl derivatives were obtained in moderate to high yields.

The chlorosulfonic derivatives of *N*-alkyl-9(10*H*)-acridones were obtained by reaction in neat chlorosulfonic acid at 60 ºC for 24 hours under vigorous stirring in N_2 atmosphere [33]. The yellow solution was then neutralized with a saturated solution of $NaHCO_3$ and the desired product extracted with chloroform obtaining the chlorosulfonic derivatives with yields ranging 28.0-69.9%. It was not possible to obtain the chlorosulfonic derivative of *N*-octyl-9(10H)-acridone by chlorosulfonation using different reaction conditions, in all cases a black solid insoluble in organic solvents and water was obtained.

The hydrolyses of the sulfonyl chloride acridones performed by refluxing in water during 48 h yield the water-soluble sulfonic acids in quantitative yields, Scheme 2.
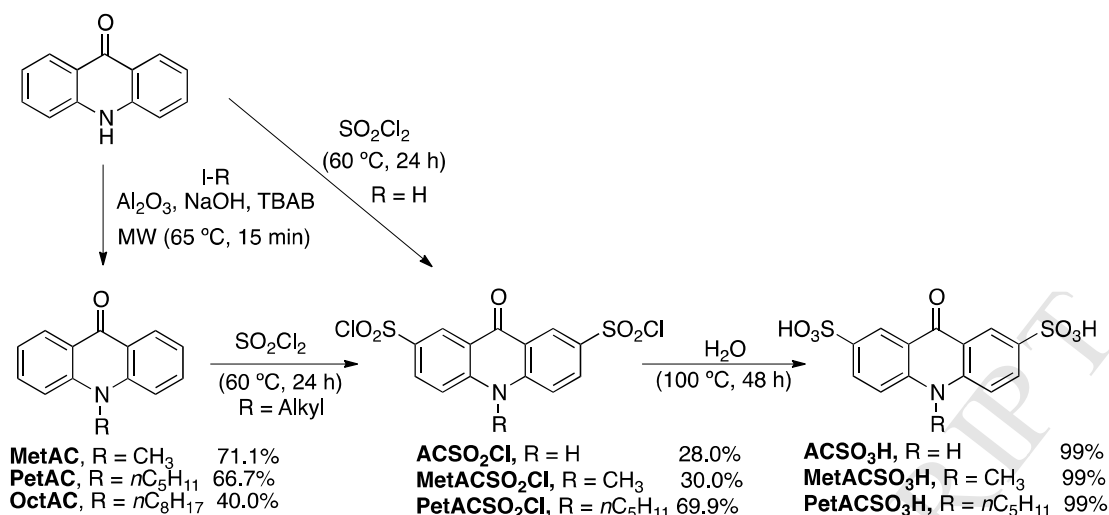
Scheme 2: Schematic reaction pathways and reaction yield for the synthesis of the *N*-alkylacridones, sulfonyl chloride acridone derivatives and the water-soluble acridone sulfonic acid derivatives.

Spectral and Photophysical data

Figures 1 and 2 depict respectively the absorption and fluorescence spectra of the three families of compounds, the (i) *N*-alkylacridones, the (ii) chlorosulfonic and (iii) sulfonic acid derivatives. The electronic spectral properties are summarized in Table 1.

The introduction of the alkyl chain in AC, ACSO$_2$Cl and ACSO$_3$H lead to the red shift of the absorption wavelength maxima in DMSO of *ca.* 5 nm, regardless the length of the chain, and to an increase of the molar extinction coefficient. In water, the same effect can be observed for AC and ACSO$_3$H derivatives with an increase of the red shift from 7 to 9 nm. Introduction of the chlorosulfonic and sulfonic acid substituents does not affect the absorption wavelength maxima but has a significant effect in the molar extinction coefficient decreasing from 9900 mol^{-1}dm^{3}cm^{-1} for AC to 6000 mol^{-1} dm^3 cm^{-1} for ACSO$_3$H.
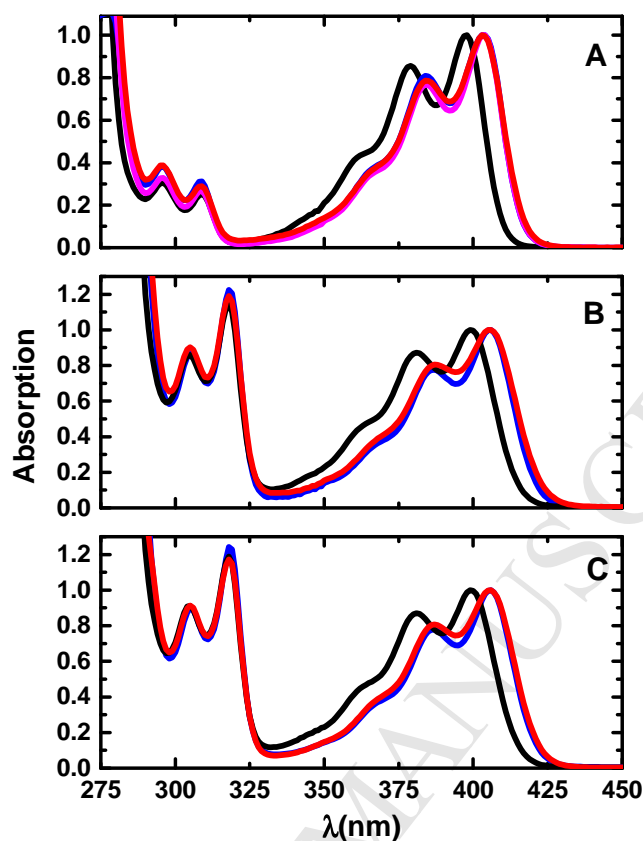
Figure 1: Absorption spectra of Acridone and its chlorosulfonic and sulfonic acid derivatives in DMSO at T=294K; **A**) black line: AC, red line: MetAC, blue line: PetAC, magenta line: OctAC. **B**) black line: $ACSO_2Cl$, red line: $MetACSO_2Cl$, blue line: $PetACSO_2Cl$. **C**) black line: $ACSO_3H$, red line: $MetACSO_3H$, blue line: $PetACSO_3H$.

The fluorescence emission maximum in DMSO displays a red-shift with the introduction of the alkyl chain, higher for *N*-methylacridone leading to also an increase in the Stoke's Shift value. This effect is further intensified on going from the less polar DMSO (solvent's permittivity $\varepsilon^{'}$=47.24) to the more polar solvent water ($\varepsilon^{'}$=78.54) achieving Stoke's shift values of ~ 2000 cm^{-1}. A less pronounced effect is observed with the chlorosulfonic acid derivatives, Table 1.
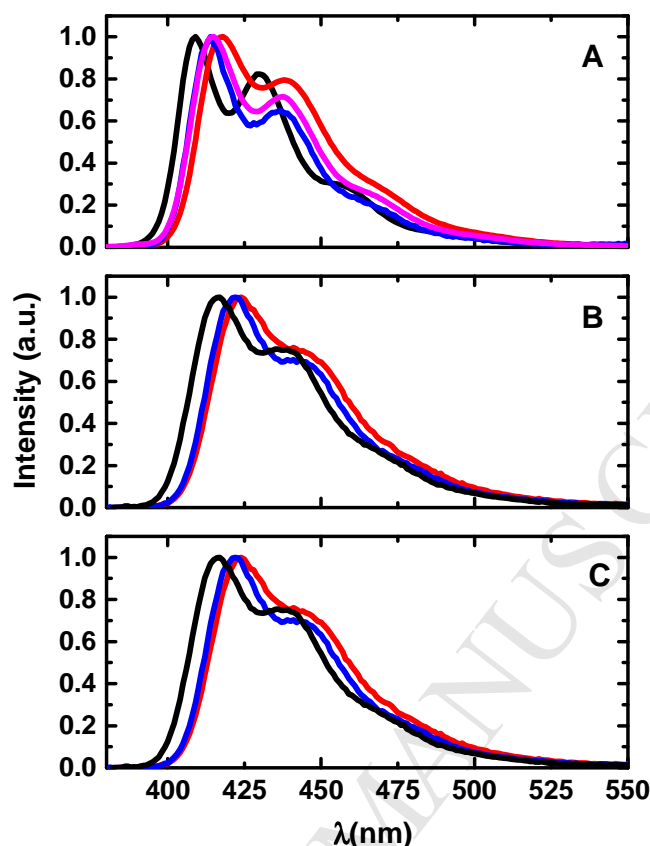
Figure 2: Emission spectra of AC and its derivatives in DMSO at T= 294 K. **A)** black line: AC, red line: MetAC, blue line: PetAC, magenta line: OctAC. **B)** black line: $ACSO_2Cl$, red line: $MetACSO_2Cl$, blue line: $PetACSO_2Cl$. **C)** black line: $ACSO_3H$, red line: $MetACSO_3H$, blue line: $PetACSO_3H$.

Table 1: Spectroscopic data including absorption (λ^{abs}(nm)) and fluorescence emission (λ^{fluo}(nm)) wavelength maxima, Stokes Shift (Δ_{SS}) and molar extinction coefficients (ε) obtained for the investigated Acridones and its chlorosulfonic and sulfonic acid derivatives in DMSO and Water (pH=5.5) at T=293 K. The dielectric constant of the solvents (ε ') is also presented.

Compound	Solvent	ε '(25º C)	λ^{abs}(nm) 293 K	λ^{fluo}(nm) 293 K	Δ_{SS} (nm)	Stokes Shift (cm^{-1})	ε(L.mol^{-1}.cm^{-1})
AC	DMSO	47.24	398	408	10	616	9900
	H_2O	80.4	385	416	31	1936	-
MetAC	DMSO	47.24	403	417	14	833	10490
	H_2O	80.4	394	430	36	2125	-

PetAC	DMSO	47.24	403	414	11	659	11290
	H_2O	80.4	394	428	34	2016	-
OctAC	DMSO	47.24	404	414	10	598	11680
	H_2O	80.4	ND	ND	ND	ND	ND
ACSO$_2$Cl	DMSO	47.24	399	417	18	1082	7240
MetACSO$_2$Cl	DMSO	47.24	406	424	18	1046	8690
PetACSO$_2$Cl	DMSO	47.24	406	422	16	934	8420
ACSO$_3$H	DMSO	47.24	399	417	18	1082	6000
	H_2O	80.4	396	417	21	1272	4430
MetACSO$_3$H	DMSO	47.24	405	424	19	1106	7490
	H_2O	80.4	403	433	30	1719	5610
PetACSO$_3$H	DMSO	47.24	406	421	15	878	7400
	H_2O	80.4	406	431	25	1429	6190

ND= not determined due to the poor solubility of this compound in water.

Photophysical Properties

The quantum yield (ϕ_F) and lifetime (τ_F) of fluorescence together with the radiative (k_F) and radiationless (k_{NR}) rate constants at T= 293 K, obtained for the investigated acridones (AC, MetAC, PentAC, and OctAC) and its chlorosulfonic (ACSO$_2$Cl, MetACSO$_2$Cl and PetACSO$_2$Cl) and sulfonic acid (ACSO$_3$H, MetACSO$_3$H and PetACSO$_3$H) derivatives in DMSO and water (pH=5.5, only for the water soluble derivatives) are summarized in Table 2.

The introduction of the alkyl group in the acridone ring influences the fluorescence quantum yield, which decreases from 0.75 (AC) to 0.65, 0.68 and 0.66 for the methyl penthyl and octyl substituted acridone respectively. The fluorescence lifetime also shows a

gradual decrease with the increase of the alkyl chain. The clorosulfonic and sulfonic acid derivatives display a longer lifetime in DMSO increasing the fluorescence quantum yield up to 0.70 (PetACSO$_3$H).
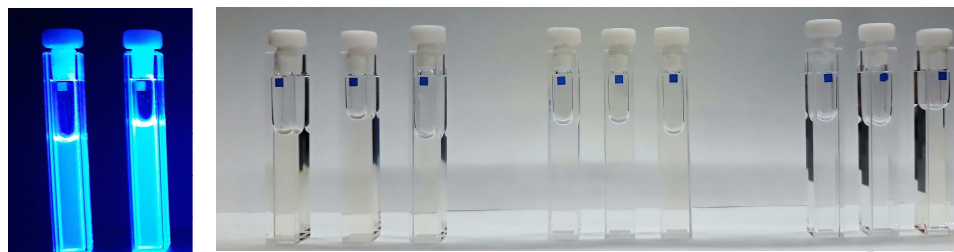

Figure 3- Picture of the studied compounds obtained under day light exposure (top picture) and (bottom picture) under UV light (365 nm). On the left ACSO$_2$Cl, MetACSO$_2$Cl and PetACSO$_2$Cl in DMSO. In the middle ACSO$_3$H, MetACS$_3$H, PetACSO$_3$H in DMSO and on right ACSO$_3$H, MetACS$_3$H and PetACSO$_3$H in water (pH=5.5).

Solubilization in water increases the fluorescence quantum yield up to 0.8 (0.82 for ACSO$_3$H and 0.92 for PetACSO$_3$H) more than 10% comparing with the fluorescence in DMSO, and also increases the decay time values; these are longer than 15 ns (16.49 ns for ACSO$_3$H and 15.62 ns for PetACSO$_3$H). It is also worth noting the constancy of the radiative (k_F) and radiationless (k_{NR}) rate constants for all the compounds and solvents. This shows that although the water-soluble acridones (ACSO$_3$H, MetACSO$_3$H and PetACSO$_3$H) display longer lifetimes the balance between the radiative and radiationless channels is kept constant and so is the dominant deactivation mechanism. Moreover, there is also a significant difference between the value in DMSO *vs*. water, showing that the compounds are sensitive to the media (polarity and pH) and adequate to use this compounds as lifetime fluorescence probes, Table 2. Although the mechanism behind this effect has not been investigated in detail in this work, it is likely that it involves the close proximity between two excited singlet excited states of S$_1$ (π,π^*) and S$_2$ (π,π^* with a contribution of an n,p* state) in which the S$_2$ becomes destabilized in water (relative to DMSO) and the S$_1$ becomes more allowed in nature.[5]

The different decay time of the water-soluble acridones (ACSO$_3$H, MetACSO$_3$H and PetACSO$_3$H) in water *vs*. DMSO lead us to further explore the pH dependence behaviour of these compounds.

Table 2: Photophysical parameters including the fluorescence quantum yield (ϕ_F) and fluorescence lifetime (τ_F) together with the radiative (k_F)and radiation less (k_{NR}) rate constants obtained for the investigated Acridones and its chlorosulfonic and sulfonic acid derivatives in DMSO and Water (pH=5.5) at T=293 K.

Compound	Solvent	ϕ_F 293 K	τ_F (ns) 293K	k_F(ns^{-1})*	k_{NR} (ns^{-1})*
AC	DMSO	0.75	9.10	0.082	0.027
MetAC	DMSO	0.65	8.85	0.073	0.040
PetAC	DMSO	0.68	8.10	0.084	0.040
OctAC	DMSO	0.66	7.96	0.083	0.043

Compound	Solvent	ϕ_F 293 K	τ_F (ns) 293K	k_F(ns^{-1})	k_{NR} (ns^{-1})
ACSO$_2$Cl	DMSO	0.70	10.78	0.065	0.028
MetACSO$_2$Cl	DMSO	0.70	10.59	0.066	0.028
PetACSO$_2$Cl	DMSO	0.76	10.08	0.075	0.024

Compound	Solvent	ϕ_F 293 K	τ_F (ns) 293K	k_F(ns^{-1})	k_{NR} (ns^{-1})
ACSO$_3$H	DMSO	0.66	10.95	0.060	0.031
	H$_2$O	0.82	16.49	0.050	0.011
MetACSO$_3$H	DMSO	0.64	10.64	0.060	0.034
	H$_2$O	0.76	16.71	0.045	0.014
PetACSO$_3$H	DMSO	0.70	10.17	0.069	0.029
	H$_2$O	0.92	15.62	0.059	0.005

$$* \ k_F = \frac{\phi_F}{\tau_F} \ ; \ k_{NR} = \frac{1-\phi_F}{\tau_F}$$

The spectra and data are summarized in Figure 4 and Table 5 respectively. Figure 4 shows a clear shift of the wavelength maxima and shape upon going from pH=0 (and 6.9) to alkaline pH (~13) values. Indeed, whereas from pH=0 to 6.9 the absorption spectra is basically identical at the two pH values, there is a small increase of the vibronic resolution at pH=6.9. In addition the energy of the first singlet excited state, S_1, (E_{0-0}) obtained from the overlap of the normalized absorption and emission spectra is identical at these two pH values ($E_{0-0}=10^7$ (408, 409 nm) = 24450 cm^{-1}, 24510 cm^{-1}) whereas at alkaline pH values (pH=13) the absorption and emission spectra together with the energy of S1 state, E_{0-0} (24969 cm^{-1}), clearly indicates the presence of a new species.
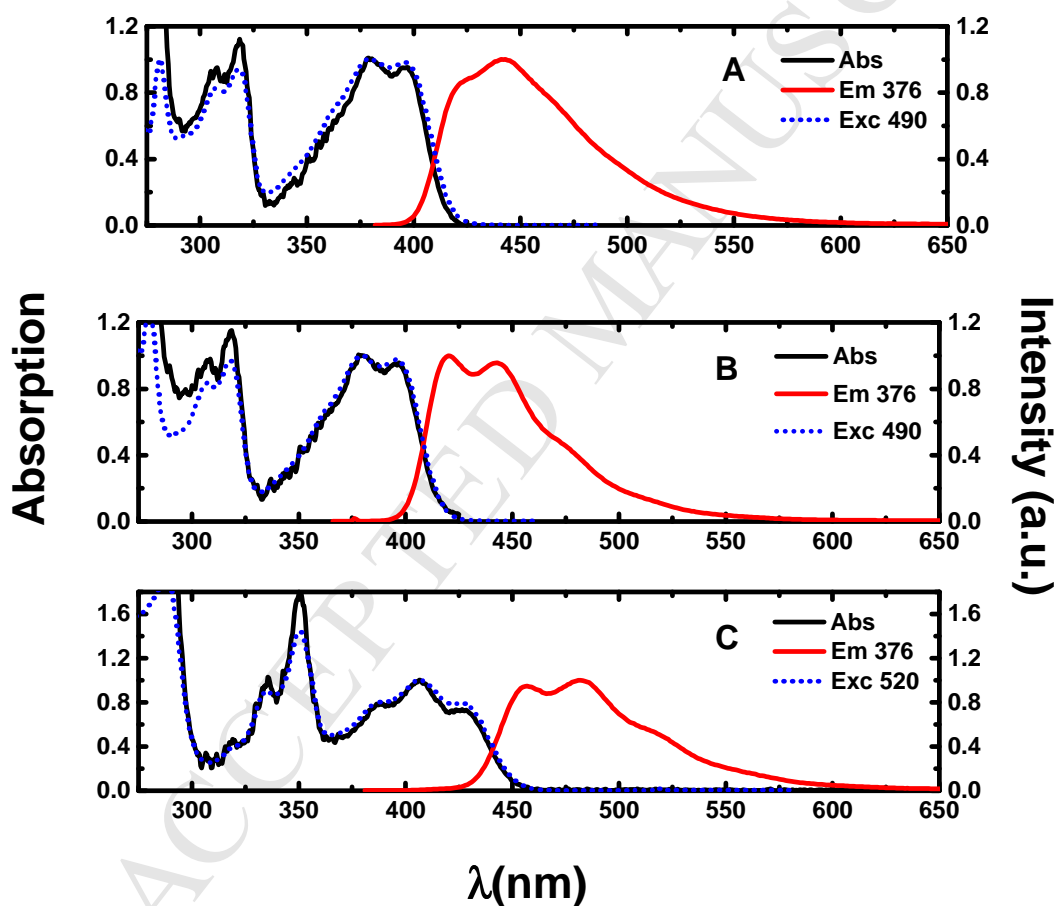

Figure 4: Absorption and emission spectra of the sulfonated Acridone $ACSO_3H$ in water at T=293 K and A) pH=0; B) pH=6.9 and C) pH=13.

However, the most interesting feature comes from the analysis of the photophysical parameters in Table 3. Indeed, as can be seen from Table 3, although the ϕ_F value is

constant with pH (exception made for the higher pH value, where the ϕ_F decreases), the same does not occur with the τ_F value thus showing that this compound can be potentially used as a lifetime probe.

Table 3–Photophysical parameters including the fluorescence quantum yield (ϕ_F) and fluorescence lifetime (τ_F) together with the radiative (k_F) and radiationless (k_{NR}) rate constants obtained for ACSO$_3$H at different pH values.

pH	ϕ_F	$\tau_{F\ (ns)}$	$k_F(ns^{-1})$	$k_{NR}\ (ns^{-1})$
0.0	0.82	18.74	0.0438	0.0096
5.5	0.81	16.28	0.0498	0.0117
6.9	0.81	17.24	0.0470	0.0110
13	0.73	22.3	0.0327	0.0121

$$k_F = \frac{\phi_F}{\tau_F}\ ;\ k_{NR} = \frac{1-\phi_F}{\tau_F}$$

Electrochemical behavior

The influence of the substituents in the acridone moitey, namely in its redox properties, was further investigated, using cyclic and differential pulse voltammetry at a glassy carbon electrode. The electrochemical behavior of the water soluble sulfonated acridones was investigated in water at different pH values. Only the 9(10*H*)-Acridone-2,7-disulfonic acid (**ACSO₃H**) presented oxidation and reduction potentials in the range -1.25 V to + 1.25 V. A 0.3 mM stock solution of the **ACSO₃H** was freshly prepared prior to use. The data obtained are summarized in Table 4.

Table 4 – Electrochemical data including the oxidation (E_{pa}) and reduction (E_{pc}) potentials for the three sulfonated acridones at three pH values. As mentioned for MetACSO$_3$H and PetACSO$_3$H no oxidation or potential could be observed in the -1.25 V to +1.25 V range. See text for more details.

Compound	E_p	
ACSO₃H	pH = 0.0	E_{pa}^1 =1.177V
	pH = 6.9	E_{pa}^1 = 1.064 V
		E_{pc}^2 = - 0.641 V
	pH = 12.8	E_{pa}^1 = 0.808 V
MetACSO₃H	-	
PetACSO₃H	-	

E_{pa}^1= oxidation potential for the first peak in the voltammogram in Fig. 4

E_{pc}^2=reduction potential for the second peak in the voltammogram in Fig. 4

The voltammetric behavior of ACSO₃H, MetACSO₃H or PetACSO₃H at a concentration of 437 µM was investigated using a GCE in a solution 0.1 M phosphate buffer solution (saturated with N₂) at pH 6.9. The cyclic voltammograms obtained for ACSO₃H at a scan rate υ = 100 mV s⁻¹, shows a single peak in the anodic region (E_{pa}^1) at 1.064 V (Figure 4); no peaks were observed in the cathodic region, indicating that the oxidation processes are irreversible. Neither MetACSO₃H nor PetACSO₃H showed redox activity thus indicating the involvement of the NH group in the redox process.
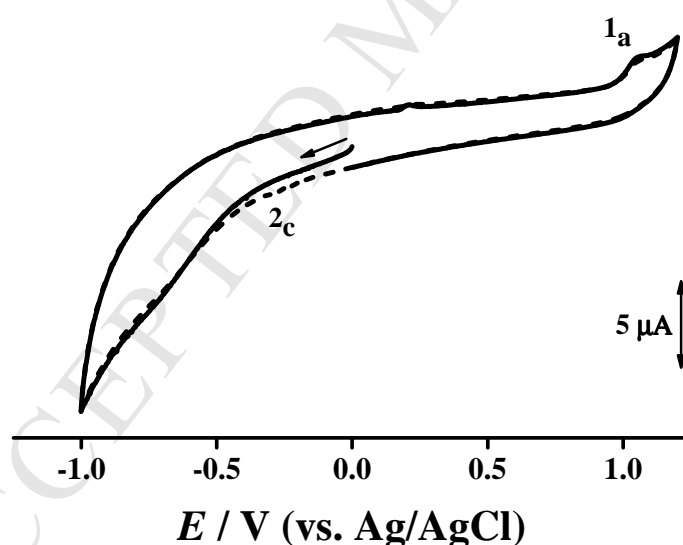

Figure 4 - Cyclic voltammograms of a solution of ACSO₃H(0.3 mM) at pH 7 (0.1 M phosphate buffer): A) (—) first and (·····) second scan, υ =100 mV s⁻¹.

On running the second CV, obtained from the ACSO₃H solution at pH= 6.9, without cleaning the GCE surface, a new reduction peak (indicated as 2c in Fig. 4), at E_{pc}^2= - 0.641 V appeared. This peak is related to the irreversible reaction of the ACSO₃H oxidation product formed at the GCE surface during the first scan. The decrease of the

current of peak 1a observed when the second scan finishes is explained by the adsorption of ACSO$_3$H and of its oxidation products at the electrode surface.

The oxidation of ACSO$_3$H is linearly pH-dependent (variation of peak 1a potential with pH), giving rise to the equation E_{pa} (V) = 0.931 – 0.059 pH. The slope of the line, 59 mV per pH unit, indicates that the same number of proton and electron are involved in the oxidation process of ACSO$_3$H. [43]

Furthermore, the number of electrons transferred, n, was determined from the peak width at half height, $W_{1/2}$ = 91 mV, and is close to the theoretical value of 90 mV, corresponding to an electrochemical reaction involving the transfer of a single electron [43]. Consequently, it can be concluded that the oxidation process for ACSO$_3$H above described involves the simultaneous transfer of one electron and one proton.

The energies of the HOMO and LUMO levels were obtained for ACSO$_3$H at different pH values from equations 1 and 2. The energy of the HOMO, E_{HOMO}, was calculated from the onset oxidation potential, $E_{onset}(ox)$, plus 4.8, which is the reference energy level of ferrocene below the vacuum level and the oxidation potential of ferrocene, E_{FOC}, given by the potential of FOC/FOC$^+$*vs* Ag/AgCl measured by cyclic voltammetry at each pH value. The LUMO energy, E_{LUMO}, was obtained from the optical energy gap E_{0-0}, measured from the interception of the normalized UV-Vis absorption and fluorescence spectra, through equation 2 at each pH value. The data are summarized in Table 5.

$$E_{HOMO} = -(E_{ox}^{onset} + 4.8) - E_{FOC} \quad (1)$$

$$E_{LUMO} = E_{HOMO} + E_{0-0} \quad (2)$$

Table 5 –Experimental parameters needed to obtain the HOMO and LUMO energy values for ACSO$_3$H at different pH values obtained from eq. (1) and (2) respectively.

pH	$E_{onset}(ox)$ (V)	E_{FOC}(V)	E_{HOMO} (eV)[c]	λ_{0-0} (nm)[a]	E_{0-0} (eV)[b]	E_{LUMO} (eV)
0.0	1.100	0.395	-5.505	409	3.031	-2.474
6.9	1.000	0.224	-5.576	408	3.039	-2.537
12.8	0.696	0.402	-5.094	400	3.099	-1.995

[a]Wavelength of the 0-0 electronic transition resulting from the intersection of the normalized absorption and the emission spectra.

[b]E_{0-0} values resulting from the intersection of the normalized absorption and the emission spectra.

Although we have developed a way to make acridones water soluble, the fact that the compounds show irreversible waves is an obvious limitation for Redox Flow Batteries (RFB) applications. Further substitution on the sulfonated acridones may induced the desired reversibility for RFB. However, the study of $ACSO_3H$ at different pH indicates that the LUMO energy levels are, in absolute value, higher than the TiO_2 conduction-band (CB) value (-4.26 eV[44], with values ranging from –4.0 to –4.3 eV[45, 46]) and could therefore make efficient organic dye photosensitizers for dye sensitized solar cells (DSSCs) since they have enough driving force for an effective injection into the CB of TiO_2.

CONCLUSIONS

A comprehensive investigation of the electronic spectral, photophysical and electrochemical properties of three different families of acridone derivatives has been undertaken in organic solvents and aqueous solutions at different pH values. Although stables when submitted to various electrochemical cycles, due to the lack of irreversible waves the compounds show limitation for RFB uses. Nonetheless, the present work shows that these compounds are highly fluorescent and have their photophysical parameters dependent on the polarity and pH values of the media. In particular with the water-soluble $ACSO_3H$ it is shown that its long fluorescent decay time value, which is shown dependent on the pH, can constitute a lifetime probe both for *in vitro* and *in vivo* studies.

ACKNOWLEDGEMENTS

REFERENCES

[1] Pal P, Zeng H, Durocher G, Girard D, Giasson R, Blanchard L, et al. Spectroscopic and photophysical properties of some new rhodamine derivatives in cationic, anionic and neutral micelles. Journal of Photochemistry and Photobiology A: Chemistry. 1996;98(1):65-72.

[2] Richardson JH, Steinmetz LL, Deutscher SB, Bookless WA, Schmelzinger WL. Measurement of Fluorescence Lifetimes of Coumarin Laser Dyes with a Mode-Locked Krypton Ion Laser. Zeitschrift für Naturforschung A1978. p. 1592.

[3] Agbavwe C, Somoza MM. Sequence-Dependent Fluorescence of Cyanine Dyes on Microarrays. PLOS ONE. 2011;6(7):e22177.

[4] Burghart A, Kim H, Welch MB, Thoresen LH, Reibenspies J, Burgess K, et al. 3,5-Diaryl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) Dyes: Synthesis, Spectroscopic, Electrochemical, and Structural Properties. The Journal of Organic Chemistry. 1999;64(21):7813-9.

[5] Seixas de Melo J, Becker RS, Elisei F, Macanita AL. The photophysical behavior of 3-chloro-7-methoxy-4-methylcoumarin related to the energy separation of the two lowest-lying singlet excited states. Journal of Chemical Physics. 1997;107(16):6062-9.

[6] Seixas de Melo J, Fernandes PF. Spectroscopy and photophysics of 4-and 7-hydroxycoumarins and their thione analogs. J Mol Struct. 2001;565:69-78.

[7] Sarder P, Maji D, Achilefu S. Molecular Probes for Fluorescence Lifetime Imaging. Bioconjugate Chemistry. 2015;26(6):963-74.

[8] Smith JA, West RM, Allen M. Acridones and Quinacridones: Novel Fluorophores for Fluorescence Lifetime Studies. Journal of Fluorescence. 2004;14(2):151-71.

[9] P. Kumar SS, D. Prosad. Acridones: A Relatively Lesser Explored Heterocycle. Key Heterocycle Cores for Designing Multitargeting Molecules: Elsevier; 2018.

[10] Akanitapichat P, Bastow KF. The antiviral agent 5-chloro-1,3-dihydroxyacridone interferes with assembly and maturation of herpes simplex virus. Antiviral Res. 2002;53(2):113-26.

[11] Sepulveda CS, Fascio ML, Garcia CC, D'Accorso NB, Damonte EB. Acridones as antiviral agents: synthesis, chemical and biological properties. Curr Med Chem. 2013;20(19):2402-14.

[12] Nguyen HT, Lallemand MC, Boutefnouchet S, Michel S, Tillequin F. Antitumor psoropermum xanthones and sarcomelicope acridones: privileged structures implied in DNA alkylation. J Nat Prod. 2009;72(3):527-39.

[13] Denny WA. Acridine derivatives as chemotherapeutic agents. Curr Med Chem. 2002;9(18):1655-65.

[14] Saito Y, Hanawa K, Kawasaki N, Bag Subhendu S, Saito I. Acridone-labeled Base-discriminating Fluorescence (BDF) Nucleoside: Synthesis and Their Photophysical Properties. Chemistry Letters. 2006;35(10):1182-3.

[15] Valdés AFC. Acridine and Acridinones: Old and New Structures with Antimalarial Activity. Open Med Chem J. 2011;5:11-20.

[16] Anderson MO, Sherrill J, Madrid PB, Liou AP, Weisman JL, DeRisi JL, et al. Parallel synthesis of 9-aminoacridines and their evaluation against chloroquine-resistant Plasmodium falciparum. Bioorg Med Chem. 2006;14(2):334-43.

[17] Fernandez-Calienes A, Pellon R, Docampo M, Fascio M, D'Accorso N, Maes L, et al. Antimalarial activity of new acridinone derivatives. Biomed Pharmacother. 2011;65(3):210-4.

[18] Belmont P, Bosson J, Godet T, Tiano M. Acridine and Acridone Derivatives, Anticancer Properties and Synthetic Methods: Where Are We Now? Anti-Cancer Agents in Medicinal Chemistry. 2007;7(2):139-69.

[19] Boumendjel A, Macalou S, Ahmed-Belkacem A, Blanc M, Di Pietro A. Acridone derivatives: design, synthesis, and inhibition of breast cancer resistance protein ABCG2. Bioorg Med Chem. 2007;15(8):2892-7.

[20] Cholewiński G, Dzierzbicka K, Kołodziejczyk AM. Natural and synthetic acridines/acridones as antitumor agents: their biological activities and methods of synthesis. Pharmacological Reports. 2011;63(2):305-36.

[21] Nikolov P, Petkova I, Köhler G, Stojanov S. Deactivation processes and hydrogen bonding of excited N-substituted acridones. J Mol Struct. 1998;448(2):247-54.

[22] Theriault KD, Radford C, Parvez M, Heyne B, Sutherland TC. Structure-property relationship of donor-acceptor acridones - an optical, electrochemical and computational study. Phys Chem Chem Phys. 2015;17(32):20903-11.

[23] Smith JA, West RM, Allen M. Acridones and quinacridones: novel fluorophores for fluorescence lifetime studies. J Fluoresc. 2004;14(2):151-71.

[24] Bahr N, Tierney E, Reymond J-L. Highly photoresistant chemosensors using acridone as fluorescent label. Tetrahedron Letters. 1997;38(9):1489-92.

[25] Lunardi CN, Bonilha JBS, Tedesco AC. Stern–Volmer quenching and binding constants of 10-alkyl-9(10H)-acridone probes in SDS and BSA. Journal of Luminescence. 2002;99(1):61-71.

[26] Miyaji H, Sessler JL. Off-the-Shelf Colorimetric Anion Sensors. Angewandte Chemie International Edition. 2001;40(1):154-7.

[27] Doering K, Meder G, Hinnenberger M, Woelcke J, Mayr LM, Hassiepen U. A Fluorescence Lifetime-Based Assay for Protease Inhibitor Profiling on Human Kallikrein 7. Journal of Biomolecular Screening. 2008;14(1):1-9.

[28] Maltman BA, Dunsmore CJ, Couturier SCM, Tirnaveanu AE, Delbederi Z, McMordie RAS, et al. 9-Aminoacridine peptide derivatives as versatile reporter systems for use in fluorescence lifetime assays. Chemical Communications. 2010;46(37):6929-31.

[29] Chattopadhyay SK, Maitra R, Kundu I, Jana M, Mandal SK, Khuda-Bukhsh AR. Acridone–Pterocarpan Conjugate: A Hybrid Molecular Probe for Recognition of Nucleic Acids. European Journal of Organic Chemistry. 2013;2013(36):8145-53.

[30] Legg KD, Hercules DM. Electrochemically generated chemiluminescence of lucigenin. Journal of the American Chemical Society. 1969;91(8):1902-7.

[31] Sakong C, Kim SH, Yuk SB, Kim JY, Park SW, Ko MJ, et al. Synthesis of Novel Quinacridone Dyes and Their Photovoltaic Performances in Organic Dye-sensitized Solar Cells. Bulletin of the Korean Chemical Society. 2011;32(8):2553-9.

[32] Selvam R, Prakash G, Subramanian K. Tunable anchoring groups@acridone-linked triphenylamine based pendant chromophores and their effects on the photovoltaic performance as sensitizers for dye-sensitized solar cells. Rsc Advances. 2016;6(110):109054-60.

[33] Pereira RC, Pineiro M, Galvão AM, Seixas de Melo JS. Thioindigo, and sulfonated thioindigo derivatives as solvent polarity dependent fluorescent on-off systems. Dyes and Pigments. 2018;158:259-66.

[34] Costa AL, Gomes AC, Pereira RC, Pillinger M, Gonçalves IS, Pineiro M, et al. Interactions and Supramolecular Organization of Sulfonated Indigo and Thioindigo Dyes in Layered Hydroxide Hosts. Langmuir. 2018;34(1):453-64.

[35] Montalti M, Credi A, Prodi L, Gandolfi M. Handbook of Photochemistry. 3 ed. Boca Raton: CRC Presss and Taylor & Francis, 2006.

[36] Pina J, Seixas de Melo J, Burrows HD, Bilge A, Farrell T, Forster M, et al. Spectral and Photophysical Studies on Cruciform Oligothiophenes in Solution and the Solid State. The Journal of Physical Chemistry B. 2006;110(31):15100-6.

[37] Striker G, Subramaniam V, Seidel CAM, Volkmer A. Photochromicity and fluorescence lifetimes of green fluorescent protein. J Phys Chem B. 1999;103(40):8612-7.

[38] Wang C, Hang T, Zhang H. Microwave-Promoted N -Alkylation of Acridones Without Solvent. Synthetic Communications. 2003;33(3):451-6.

[39] D.D. Perrin BD. Buffers for pH and Metal Ion Control: J. Wiley, 1974.

[40] Sepúlveda CS, Fascio ML, Mazzucco MB, Palacios MLD, Pellón RF, García CC, et al. Synthesis and Evaluation of N-Substituted Acridones as Antiviral Agents against Haemorrhagic Fever Viruses. Antiviral Chemistry and Chemotherapy. 2008;19(1):41-7.

[41] Kumar R, Bahia MS, Silakari O. Synthesis, cytotoxic activity, and computational analysis of N10-substituted acridone analogs. Medicinal Chemistry Research. 2015;24(3):921-33.

[42] Kelly JX, Smilkstein MJ, Cooper RA, Lane KD, Johnson RA, Janowsky A, et al. Design, Synthesis, and Evaluation of 10-N-Substituted Acridones as Novel Chemosensitizers in *Plasmodium falciparum*. Antimicrobial Agents and Chemotherapy. 2007;51(11):4133-40.

[43] Brett CMA, and A. M. Oliveira-Brett. Electrochemistry: Principles, Methods and Aplication: Oxford: Oxford Science Publications, 1993.

[44] Chung I, Lee B, He J, Chang RPH, Kanatzidis MG. All-solid-state dye-sensitized solar cells with high efficiency. Nature. 2012;485:486.

[45] Mishra A, Fischer MKR, Bäuerle P. Metal-Free Organic Dyes for Dye-Sensitized Solar Cells: From Structure: Property Relationships to Design Rules. Angewandte Chemie International Edition. 2009;48(14):2474-99.

[46] Hara K, Sayama K, Ohga Y, Shinpo A, Suga S, Arakawa H. A coumarin-derivative dye sensitized nanocrystalline TiO2 solar cell having a high solar-energy conversion efficiency up to 5.6%. Chemical Communications. 2001(6):569-70.

Highlights

New water-soluble acridones have been synthesized

The displayed photophysical properties have shown these as potential polarity and pH probes

The electrochemical properties have shown these as potential dyes for DSSC