

Analytical Methods

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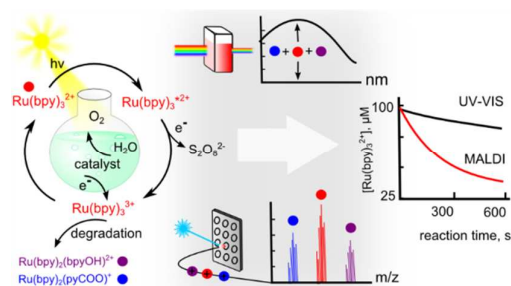
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Graphical abstract

The developed MALDI MS method for the quantitative determination of $\text{Ru}(\text{bpy})_3^{2+}$ in photooxidation reactions provides more reliable results than the wide-used spectrophotometric method.



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3 Quantitative Determination of the Ru(bpy)₃²⁺ Cation in Photochemical
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6 Reactions by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass
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10 Spectrometry
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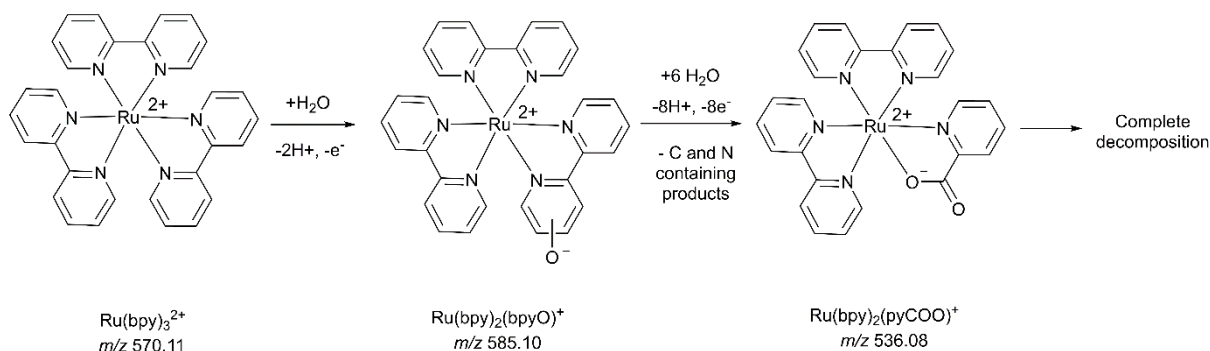
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32 Introduction

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35 The outstanding combination of ground-state stability, excited-state lifetime and reactivity,
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37 redox properties, and luminescence emission has made the tris(2,2'-bipyridine)ruthenium(II)
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39 complex one of the most employed compounds in several different research fields during
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41 the last 30 years. Electrochemiluminescent analysis,¹ photocatalytic organic synthesis,²
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43 photoinduced intermolecular electron and energy transfer³, light-emitting devices⁴,
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45 supramolecular machines⁵, and molecular computing⁶ are some representative examples of
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47 the wide utilization of Ru(bpy)₃²⁺. Considerable attention has been focused on this
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49 ruthenium complex within solar energy conversion due to its ability to drive both
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51 photooxidative (e.g., water oxidation) and photoreductive (e.g., proton or carbon dioxide
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53 reduction) reactions in the presence of the appropriate oxidative or reductive quencher,
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55 respectively.
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3 The combination of $\text{Ru}(\text{bpy})_3^{2+}$, as a photosensitizer, with the peroxydisulfate ion, $\text{S}_2\text{O}_8^{2-}$, as
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5 an electron acceptor, has been widely used in synthetic organic photochemistry as well as in
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7 light-driven water oxidation. Upon visible light illumination, a long-lived excited state of the
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9 ruthenium complex is formed. The resulting high energy species $\text{Ru}(\text{bpy})_3^{*2+}$ donates an
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11 electron to the peroxydisulfate ion. Apart from the formation of $\text{Ru}(\text{bpy})_3^{3+}$, this electron
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13 transfer causes decomposition of $\text{S}_2\text{O}_8^{2-}$ into SO_4^{2-} and $\text{SO}_4^{\bullet-}$, thus minimizing the back-
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15 electron transfer.⁷ A drawback of this system is that highly reactive products of the
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17 photoreaction, $\text{Ru}(\text{bpy})_3^{3+}$ and $\text{SO}_4^{\bullet-}$, promote oxidative degradation of coordinated
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19 bipyridine ligands.⁸ The ruthenium species formed by this degradation have lower oxidative
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21 potential⁹ and are not able to perform the photoreaction as efficient as $\text{Ru}(\text{bpy})_3^{2+}$.
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23 Eventually, when the undamaged photosensitizer is depleted, the photoreaction stops. The
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25 most frequently used method to determine the concentration of $\text{Ru}(\text{bpy})_3^{2+}$ and to follow
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27 the degradation process is UV-Vis spectroscopy. The tris(2,2'-bipyridine)ruthenium(II)
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29 complex strongly absorbs light in the visible region with a maximum at 452 nm ($\epsilon = 14600 \text{ M}$
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31 cm^{-1} in water); and UV-Vis determination is fast and simple. However, Sutin et al.⁹ showed
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33 that the degradation products (Scheme 1) absorbs light at the same region ($\lambda_{\text{max}} = 455 - 457$
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35 nm, $\epsilon = 10300 - 13000 \text{ M cm}^{-1}$). This means that UV-Vis spectroscopy might give
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37 overestimated results on the concentration of intact $\text{Ru}(\text{bpy})_3^{2+}$ in photochemical reaction
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39 mixtures.
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52 Herein, we report a novel method for quantitative analysis of $\text{Ru}(\text{bpy})_3^{2+}$ by MALDI TOF MS.
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54 We applied this method for the determination of $\text{Ru}(\text{bpy})_3^{2+}$ in a catalytic reaction of
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56 photochemical water oxidation and obtained more reliable data compared to the UV-Vis
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58 spectrometric analysis.
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Scheme 1. $\text{Ru}(\text{bpy})_3^{2+}$ degradation products suggested by Sutin and co-workers⁹ and confirmed by MALDI MS in the present work.

Experimental

Reagents and solution preparation

The equimolar exchange reaction between $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$ and AgClO_4 in water followed by recrystallization from acetonitrile/diethyl ether mixture was used for the preparation of $[\text{Ru}(\text{bpy})_3](\text{ClO}_4)_2$. The bluish-green modification of $\text{Co}(\text{OH})_2$ was obtained from aqueous solution of CoSO_4 by precipitation with 2 equivalents of NaOH . All other reagents were purchased (Aldrich) and used as received without further purification.

The following aqueous stock solutions were used in the present work: $[\text{Ru}(\text{bpy})_3](\text{ClO}_4)_2$ 1 mM, $[\text{Ru}(\text{phen})_3]\text{Cl}_2$ 1 mM, Na_2SO_4 200 mM, Na_2SO_3 20 mM, sodium phosphate buffer (pH 7) 100 mM, $\text{Co}(\text{ClO}_4)_2$ 0.1 mM, and $\text{Na}_2\text{S}_2\text{O}_8$ 100 mM.

The calibration standard and control solutions of $[\text{Ru}(\text{bpy})_3](\text{ClO}_4)_2$ were prepared from the stock solution in deionized water at concentrations of 10, 20, 40, 60, 80, 100 and 15, 50, 90 μM , respectively. Sodium phosphate buffer (1 M) and cobalt(II) hydroxide suspension (1M, 0.1 g/mL) were spiked with the $[\text{Ru}(\text{bpy})_3](\text{ClO}_4)_2$ stock solution to produce the 15, 50, and 90 μM control solutions with a high salt or $\text{Co}(\text{OH})_2$ solid particle content.

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3 The quenching solution was prepared by mixing Na₂SO₄ and Na₂SO₃ stock solutions and
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5 methanol in a volume ratio of 1:1:2, giving a final concentration of 50mM Na₂SO₄ and 5 mM
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7 Na₂SO₃. The Ru(phen)₃Cl₂ stock solution was diluted with 2-propanol yielding the internal
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9 standard (IS) solution at a concentration of 30 μM. The MALDI matrix, DCTB, was prepared at
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11 a concentration of 15 mg/mL in 2-propanol.
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16 Solutions for the MALDI experiment were prepared by mixing 10 μL of the quencher
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18 solution, 5 μL of the IS solution, 5 μL of the [Ru(bpy)₃](ClO₄)₂ calibration or control solution,
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20 and 80 μL of the matrix solution. For the MALDI experiment, 2 μL of the
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22 sample/quencher/matrix mixture was spotted onto a target plate pre-heated to 41 °C.
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26 27 28 **Collection of the samples during the photochemical reaction of water oxidation**

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30 An aqueous mixture (4 mL) containing Co(ClO₄)₂ (20 μM), [Ru(bpy)₃](ClO₄)₂ (90 μM), Na₂S₂O₈
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32 (2 mM), phosphate buffer pH 7.0 (20 mM) was exposed to visible light illumination (LEDs, λ =
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34 470±10 nm, ~820 μE/cm²·s). Samples (100 μL) were taken after 0, 10, 30, 60, 90, 120, 180,
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36 300, and 600 s of irradiation and mixed immediately with the quenching solution (200 μL).
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38 To 15 μL of the sample/quencher mixture, 5 μL of IS and 80 μL of matrix solutions were
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40 added. Then, 2 μL of the resulting sample/quencher/matrix mixtures were spotted onto a
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42 MALDI target plate pre-heated to 41 °C. The [Ru(bpy)₃]²⁺ content was quantified by MALDI
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44 MS. The rest of the sample/quencher mixture were analysed by UV/Vis spectroscopy.
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50 51 52 **Quenching solution performance tests**

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54 In the first test, the freshly prepared reaction mixture (100 μL) was mixed with the
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56 quenching solution (200 μL) and exposed to visible light illumination for 10 min. After that,
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58 the reaction mixture was analysed by MALDI MS as it is described in the previous section.
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60 Ratios between areas of the IS peak (m/z 642.11) and the [Ru(bpy)₃]²⁺ decomposition

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3 products peaks (m/z 536.08 and 585.10) were checked (Electronic supplementary
4 information (ESI), Fig. S-1).
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9 In the second test, the sample/quencher mixtures obtained after 0, 30, 60, 180, 300, and 600
10 s of the light irradiation were again analysed by MALDI MS the next day after the
11 photochemical reaction. The results of the same-day and next-day analyses were compared
12 (Fig. S-2).
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19 20 **UV-Vis spectroscopy**

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22 Light absorption at 452 nm of the solutions containing $[\text{Ru}(\text{bpy})_3]^{2+}$ was measured on UV4
23 ATI UNICAM UV/Vis spectrometer.
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28 29 **MALDI MS**

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31 The MALDI TOF MS experiments were performed in a positive reflector mode with delayed
32 extraction (150 ns) on a Bruker Daltonics MALDI Ultraflex II spectrometer equipped with a
33 pulsed N_2 laser (337 nm). The instrument settings were following: ion source I 25.0 kV, ion
34 source II 21.7 kV, lens voltage 10.1 kV, reflector voltage I 26.3 kV, reflector voltage II 13.8 kV,
35 laser attenuation 77-82 %, laser repetition rate 200 Hz. Spectra were acquired automatically
36 for the m/z range of 100 - 1000. Each spectrum was the sum of 1000 single laser shots
37 randomised over 100 positions within the same spot (10 shots/position).
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50 Nine commonly used MALDI matrices, *trans*-2-[3-(4-*tert*-Butylphenyl)-2-methyl-2-
51 propenylidene]malononitrile (DCTB), α -cyano-4-hydroxycinnamic acid (CHCA), 2,5-
52 dihydroxybenzoic acid (DHB), sinapinic acid (SA), 2,5-dihydroxyacetophenone (DHAP), 2,4,6-
53 trihydroxyacetophenone (THAP), 9-aminoacridine (AA), dithranol (Dith), and salicylamide
54 (SalAm) were examined, however, only DCTB was employed through the work. Calibration
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3 was performed externally using a mixture of CHCA (m/z 190.050, 379.093, and 568.135), tri-
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5 tyrosine (m/z 508.208), and penta-phenylalanine (m/z 754.360) as a calibration standard.
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9 A Bruker MTP 384 ground steel target plate was utilized for the MALDI MS experiments. All
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11 analyses were conducted in triplicate. To prevent considerable sublimation of the DCTB
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13 matrix inside the instrument, not more than 96 spots were analysed at one automatic run.
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15 The AutoXecute option of the FlexControl 3.0 operating software was exploited for
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17 automatic data acquisitions. FlexAnalysis method and macro, created for automatic data
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19 processing, are given in the ESI.
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25 Ruthenium compounds have a broad isotope distribution (Fig. S-3), where each singular peak
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27 or a group of peaks can be used for the quantification purpose. In this work, two methods
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29 for calculation of the analytical signal were tested. In the “one peak” approach, the
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31 analytical signal was determined as a ratio of the area under the most intensive $\text{Ru}(\text{bpy})_3^+$
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33 peak (m/z 570.11) to the area under the most intensive $\text{Ru}(\text{phen})_3^+$ peak (m/z 642.11). In the
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35 “seven peaks” approach, the analytical signal was calculated as a ratio of the area under the
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37 seven most intensive $\text{Ru}(\text{bpy})_3^+$ peaks (m/z 567.11, 568.11, 569.11, 570.11, 571.11, 572.11,
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39 and 573.11) to the area under the seven most intensive $\text{Ru}(\text{phen})_3^+$ peaks (m/z 639.11,
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41 640.11, 641.11, 642.11, 643.11, 644.11, and 645.11). Within-day reproducibility, accuracy,
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43 and linearity were calculated using both approaches (Tab. S-1 and S-2). No advantages of
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45 using the more complicated “seven peaks” method were found, therefore, the simpler “one
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47 peak” method was applied for all other calculations in this work.
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54 55 **Results and Discussion**

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58 In this work, a classical system for photocatalytic water oxidation¹⁰ was chosen as the object
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60 of the analysis. It contained $[\text{Ru}(\text{bpy})_3](\text{ClO}_4)_2$ as a photosensitizer, $\text{Na}_2\text{S}_2\text{O}_8$ as a sacrificial

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3 electron acceptor, $\text{Co}(\text{ClO}_4)_2$ as a catalyst precursor, and phosphate buffer (pH 7) to maintain
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5 acidity of the reaction mixture at a narrow range.
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8 9 **Analytical method development**

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11 High sensitivity, low sample consumption, and tolerance towards relatively high buffer and
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13 solid particle contents make MALDI MS an invaluable analytical tool for studying
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15 heterogeneous reaction mixtures. Comparing to LC MS, MALDI MS offers faster analysis. For
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17 example, LC MS analysis of ruthenium polypyridine complexes developed by Lund and co-
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19 workers¹¹ takes ≈ 40 min per sample, whereas determination of $\text{Ru}(\text{bpy})_3^{2+}$ by MALDI MS in
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21 this work requires ≈ 30 s per sample. A drawback of quantitative MALDI MS analysis is the
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23 poor shot-to-shot and batch-to-batch reproducibility, caused by the inhomogeneous co-
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25 crystallization of the analytes with the matrix. However, this issue can be straightforwardly
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27 solved by using internal standards. Stable isotope labelled internal standards provide the
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29 best results of the analysis, but they are expensive and frequently unavailable. The cheaper
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31 unlabelled compounds with similar chemical structures and properties can be employed, still
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33 providing eligible results. In this work, for the quantitative MALDI MS determination of
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35 $\text{Ru}(\text{bpy})_3^{2+}$ cation, we applied the latter approach and chose $\text{Ru}(\text{phen})_3^{2+}$ (phen - 1,10-
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37 phenanthroline) as an internal standard.
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48 Ruthenium polypyridine complexes are light-sensitive and may photodissociate under UV
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50 irradiation (Fig. S-4), therefore, a matrix requiring as low a laser fluence to desorb/ionise the
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52 ruthenium complexes as possible is needed. We found that among the regular matrices,
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54 DCTB is most suitable for the analysis of $\text{Ru}(\text{bpy})_3^{2+}$. The intensive molecular peak of the
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56 analyte (m/z 570.11) appears already at 16 – 25 % of the maximal laser power (depending on
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58 the salt concentration in the sample). At these conditions, photodissociation of $\text{Ru}(\text{bpy})_3^{2+}$ to
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3 Ru(bpy)₂⁺ (m/z 414.04) is insignificant. The area of the m/z 414.04 peak (S₄₁₄) is less than
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6 0.01 of the m/z 570.11 peak area (S₅₇₀) in the case of DCTB, whereas for the other matrices
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8 photodissociation is higher (S₄₁₄/S₅₇₀ is equal 0.1 for CHCA, SA, and DHB; 0.3 for DHAP and
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10 THAP; 4 for SalAm and Dith; and 10 for AA). Moreover, DCTB is an aprotic compound and
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12 contains no ligating groups; therefore, it does not compete with bipyridine and
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14 phenanthroline for the coordination sites of ruthenium during the MALDI experiment.
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18 However, the DCTB matrix has some limitations.¹² One of them is its water insolubility. To
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20 avoid precipitation of the matrix after mixing with the aqueous sample solution, we added
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22 excess of the 2-propanol solution of DCTB. Another weakness of DCTB is that it sublimes in
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24 the high-vacuum environment inside the MALDI instrument. This property of the matrix may
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26 affect a prolonged automated analysis. To eliminate this problem we increased the amount
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28 of the matrix on the spots of the target plate by putting 2 μL of the sample/matrix mixture.
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30 Also, we recommend the total number of samples to be suitable for the run time not
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32 exceeding one hour. According to our observations, the signal intensity obtained from one
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34 particular sample during this time remains stable through a series of consecutive analyses.
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38 The presence of Ru(bpy)₃³⁺ and SO₄^{•-} in the light-irradiated reaction mixture may distort the
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40 results of the determination since these products of the photochemical reaction continue to
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42 promote the degradation of bipyridine during the sample preparation step. In addition, the
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44 above-mentioned highly oxidative species appear in the samples containing unreacted S₂O₈²⁻,
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46 if these samples are handled under room light conditions. Therefore, pretreatment of the
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48 samples taken during the course of the photochemical reaction by a “quenching” solution,
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50 which reduces Ru(bpy)₃³⁺, quenches SO₄^{•-}, and decomposes unreacted S₂O₈²⁻, is required. A
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52 wide-used reductant as the iron(II) cation and a radical quencher such as ascorbic acid both
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54 diminish the intensities of the analyte and internal standard signals and overcomplicate the
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3 MALDI spectra. In contrast, we found that a mixture of 10 mM aqueous solution of Na_2SO_3
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5 with methanol (1:1, v/v) efficiently stops the $\text{Ru}(\text{bpy})_3^{2+}$ degradation processes.
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10 We established that the response factor ($K = (S_{571}/S_{642})([\text{Ru}(\text{phen})_3^{2+}]/[\text{Ru}(\text{bpy})_3^{2+}])$) depends
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12 on the $[\text{SO}_3^{2-}]/[\text{SO}_4^{2-}]$ ratio in the sample. Therefore, in order to buffer the SO_4^{2-}
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14 concentration and keep the K value constant, an excess of Na_2SO_4 was added to the
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16 quenching solution giving its final concentration of 50 mM.
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21 Two types of tests were performed to show the effectiveness of the sulphite-methanol
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23 “quenching” solution. In the first test, one volume of the photochemical reaction mixture
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25 was added to two volumes of the quenching solution and irradiated with 470 nm light for 10
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27 minutes. Only a low-intensity signal at m/z 585.10 ($S_{585}/S_{642} = 0.14$), which corresponds to
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29 $\text{Ru}(\text{bpy})_2(\text{bpyO})^+$, was detected (Fig. S-1A). The peak at m/z 536.08 corresponding to
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31 $\text{Ru}(\text{bpy})_2(\text{pyCOO})^+$ did not appear at all. Whereas, in the absence of the quenching solution,
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33 much more intensive peaks of the side products ($S_{585}/S_{642} = 0.75$ and $S_{536}/S_{642} = 0.31$) were
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35 observed (Fig. S-1B). The result of this experiment indicates that the quenching solution
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37 efficiently breaks off the light-driven interaction between $\text{Ru}(\text{bpy})_3^{2+}$ and $\text{S}_2\text{O}_8^{2-}$ and makes
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39 the treated samples light insensitive. In the second test, the samples taken during the course
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41 of the photochemical reaction were treated with the quenching solution. The obtained
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43 sample/quencher mixtures were analysed straight away and the next day (Fig. S-2). The
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45 results of the inter-day MALDI MS determinations of $\text{Ru}(\text{bpy})_3^{2+}$ match each other, and the
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47 relative average deviations do not exceed 12 %. This demonstrates that the proposed
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49 quenching solution serves as an effective preservative.
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Method Validation

Usually, the initial concentration of $\text{Ru}(\text{bpy})_3^{2+}$ in photochemical reactions falls within the range of 1.5 – 0.1 mM. We focused our study on the lower border of this interval. The IS was used at the concentration of 30 μM . The limit of detection for the proposed assay was found to be 1 μM . However, accuracy and RSD for the control samples with the concentration of $\text{Ru}(\text{bpy})_3^{2+}$ less than 10 μM were more than 20%. We obtained good linearity ($R^2 = 0.968 - 0.997$), RSDs ($< 15.4\%$), and accuracy ($< 14.5\%$) in the quantified region between 100 and 10 μM (Tables 1, S-1, and S-3). This means that the level of the $\text{Ru}(\text{bpy})_3^{2+}$ degradation can be detected with good precision in the range from 0 to 90 %.

To understand whether the presence of salt or solid particles affects the performance of the present method, we applied it for the determination of $\text{Ru}(\text{bpy})_3^{2+}$ in 1 M phosphate buffer and 1M (equal to 0.1g/mL) suspension of $\text{Co}(\text{OH})_2$. The obtained results summarised in Table 1 show that a high salt or particles content does not hinder the MALDI MS analysis of $\text{Ru}(\text{bpy})_3^{2+}$.

Table 1. The analytical parameters of the proposed MALDI MS quantification of $\text{Ru}(\text{bpy})_3^{2+}$.

[$\text{Ru}(\text{bpy})_3^{2+}$] (μM)	Within-day		Between-day		1M phosphate		1M $\text{Co}(\text{OH})_2$	
	RSD (%)	accuracy (% error)	RSD (%)	accuracy (% error)	RSD (%)	accuracy (% error)	RSD (%)	accuracy (% error)
90	2.0 – 15.4	3.2 – 12.4	3.4 – 15.4	3.2 – 14.5	2.7	5.5	3.8	6.5
50	2.5 – 8.5	2.3 – 12.3	1.8 – 12.3	2.8 – 9.7	2.1	4.3	9.6	11.9
15	6.7 – 8.6	0.1 – 9.9	4.4 – 9.3	0.1 – 13.7	6.5	3.9	13.2	9.3

Comparison with UV-Vis spectroscopic determination

A series of samples were taken from the exposed to light reaction mixture. The samples were analysed by the proposed MALDI MS method as well as by UV-Vis spectroscopy. It can be seen from Fig. 1 that the difference between the values obtained by mass-spectrometry and spectrophotometry becomes larger as the photochemical reaction carries on. Mass

spectrometric analysis shows that the level of $\text{Ru}(\text{bpy})_3^{2+}$ decomposition reaches 68 % after 600 s of light irradiation. However, the results of UV-Vis spectroscopy indicate that only 16 % of $\text{Ru}(\text{bpy})_3^{2+}$ has decayed at that point.

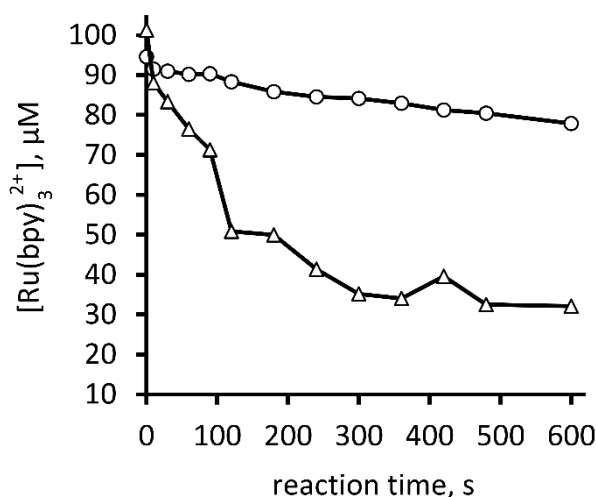


Figure 1. Concentration of $\text{Ru}(\text{bpy})_3^{2+}$ during the course of the photochemical reaction determined by UV-Vis (\circ) and MALDI MS (Δ).

This contradiction can be explained with the help of the mass spectra shown in Fig. 2. They indicate an appearance of other Ru species in the reaction mixture under the LED illumination. The peaks at m/z 536.08 and 585.10 (Fig. 2B), which are absent in the spectrum of the reaction mixture before exposure to light (Fig. 2A), can be assigned to $\text{Ru}(\text{bpy})_2(\text{bpyO})^+$ and $\text{Ru}(\text{bpy})_2(\text{pyCOO})^+$. The formation of these ruthenium species is in agreement with the speculative mechanism of $\text{Ru}(\text{bpy})_3^{2+}$ degradation suggested by Sutin and co-workers.⁹ Consequently, UV-Vis spectroscopy gives the total concentration of the ruthenium complexes in the reaction mixtures, whereas MALDI MS distinguishes between those compounds and allows researchers to analyse each ruthenium species separately.

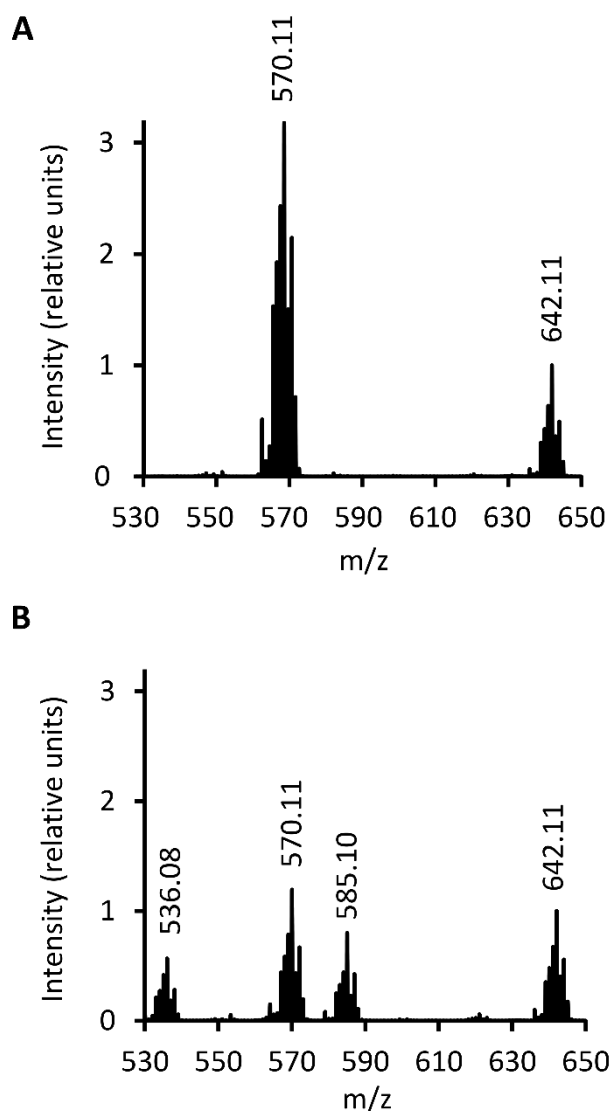


Figure 2. MALDI spectra of samples taken from the reaction mixture before (A) and after 10 min of irradiation (B). The additional peaks at m/z 536.08 and 585.10, which appear in spectra of light-irradiated samples, can be assigned to the $\text{Ru}(\text{bpy})_3^{2+}$ degradation products - $\text{Ru}(\text{bpy})_2(\text{bpyO})^+$ and $\text{Ru}(\text{bpy})_2(\text{pyCOO})^+$, respectively. Signal intensities were normalized to the peak at m/z 642.11 corresponding to the IS.

The correct information about the content of intact $\text{Ru}(\text{bpy})_3^{2+}$ in the photocatalytic reaction mixture is vital for the development of catalysts for light-driven water oxidation. As reported by Sutin and co-workers, the degradation products (their exact compositions were not established) possess lower $\text{Ru}^{\text{III/II}}$ potential than $\text{Ru}(\text{bpy})_3^{2+}$. Indeed, according to Åkerman

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3 and co-workers,¹³ the Ru^{III/II} potential for synthetic Ru(bpy)₂(pyCOO)⁺ is equal to + 0.88 V vs
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6 SCE in acetonitrile (+0.83 V vs NHE in water). This is less than the 1.30 V (NHE) required for
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8 water oxidation on the cobalt oxide catalyst (0.82 V of O₂/H₂O thermodynamic potential at
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10 pH 7 plus 0.48 V of overpotential).¹⁴ This means that only the initial Ru(bpy)₃²⁺ can drive the
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12 photocatalytic water oxidation. Overestimation of the concentration of the unimpaird
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14 photosensitizer at the end of the reaction may lead to the misinterpretation that the
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16 photosensitizer at the end of the reaction may lead to the misinterpretation that the
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18 photocatalytic reaction stops due to deactivation of the catalyst, while it is instead the
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20 photosensitizer that has been destroyed.
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24 We believe that MALDI MS has a great potential to become a powerful analytical tool for the
25
26 solar fuel research. Usually, solar energy converting systems consist of a photosensitizer and
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28 a catalyst co-adsorbed on the surface of metal oxide (TiO₂,¹⁵ NiO¹⁶). Such an architecture is
29
30 perfect for laser desorption/ionization mass spectrometric analysis, because both the metal
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32 oxide and the photosensitizer absorb UV-laser irradiation. In the case, when the matrix-free
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34 approach is not efficient, organic matrix can be always added to support desorption
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36 ionization processes. Moreover, MALDI MS analysis can be conducted directly on the surface
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38 of metal oxide and, in contrast to LC-MS analysis,¹⁷ does not require an extraction
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40 preliminary step.
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46 47 48 **Conclusions**

49
50 The performed studies show that MALDI MS can be successfully employed for the
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52 quantitative analysis of Ru(bpy)₃²⁺ in heterogeneous reaction mixtures with a high salt
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54 content. An advantage of MALDI MS quantification of the photosensitizer in the
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56 photocatalytic reaction of water oxidation in comparison with the widely used UV-Vis
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58 spectroscopy was demonstrated. The spectrophotometric method reveals only the total
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3 concentration of all ruthenium complexes with bipyridine and its derivatives, since they all
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5 have essentially the same absorption maxima, whereas the mass spectrometry assay
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7 provides separate information on these species.
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11 Electronic supplementary information (ESI) is available. See DOI:
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13

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