

# An Electrospray Ionization Source for the Investigation of Thermally Initiated Reactions

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**An electrospray ionization source for mass spectrometry is described, which allows one to heat the spray capillary. Thermal dissociation of the trityl dimer 1 and tetra(*p*-anisyl)hydrazine (3) have been investigated, operating the ESI source as electrolytic cell to ionize neutral species, e.g. the trityl radical 2.**

Electrospray ionization (ESI) mass spectrometry (MS), introduced by Fenn et al.<sup>1</sup> and Alexandrov et al.,<sup>2</sup> has been shown to be useful for analysis of various kinds of analytes. With this technique, it became possible to analyze reaction substrates, products, and intermediates at the same time directly from reaction solutions. There are some examples in the literature that deal with organic reactions at room temperature,<sup>3–11</sup> very few operating at temperatures below room temperature,<sup>12</sup> but we found no publications that are concerned with the ESI investigation of reactions at elevated temperatures. As our work has been focusing on radical reactions<sup>13</sup> and many radical reactions can be initiated by thermal decomposition of, for example, azo compounds or peroxides at elevated temperatures, we developed an ESI source that allows one to heat the reaction solution only a short time before the ionization occurs, or (even better) to heat the ionization region, thus enabling us to detect thermally generated reactive intermediates with very low steady-state concentration in the

reaction solution. Because radicals are neutral species and are not ionized in the ESI process in general, we thought to operate the ESI source as an electrolytic cell as described by Van Berkel.<sup>14</sup> Thus, the thermally generated radicals should hopefully be oxidized electrolytically in situ to cations that can be observed by mass spectrometry.

As first examples, we have investigated the behavior of the trityl dimer (1-diphenylmethylene-4-trityl-2,5-cyclohexadiene) (1) and of tetra(*p*-anisylhydrazine (3) at different temperatures.

## EXPERIMENTAL SECTION

**Mass Spectrometry.** All experiments were performed on a Finnigan LCQ (Thermoquest-Finnigan, San Jose, CA) quadrupole ion trap mass spectrometer equipped with a home-built ESI ion source (vide infra). Sample solutions ( $\sim 5 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ ) were infused using the syringe pump of the LCQ. ESI operation conditions involved a spray voltage of +2–2.5 kV, a heated capillary temperature of 50 °C, and a flow rate of  $2 \mu\text{L}\cdot\text{min}^{-1}$ . The mass spectrometer was operated in full-scan profile mode, scanning from  $m/z$  50 to 500 at unit resolution. Data acquisition and analysis was done with the Xcalibur (version 1.0, Thermoquest-Finnigan) software package.

**Ion Source.** Our ion source is based on a design developed by Wang and Hackett.<sup>15</sup> It consisted of a fused-silica spray capillary (100- $\mu\text{m}$  i.d., 200- $\mu\text{m}$  o.d., Polymicro, Phoenix, AZ) in which a platinum wire (50- $\mu\text{m}$  diameter, Goodfellow, Huntingdon, U.K.) was inserted. Details are shown in Figure 1. The capillary was connected to a PEEK cross (Upchurch, Oak Harbor, WA) with a Teflon sleeve (VICI-Jour Research, Onsala, Sweden), while the platinum wire traversed the cross (for details, see Figure 2) and was sealed by a Teflon sleeve (150- $\mu\text{m}$  i.d., Valco, Schenkon, Switzerland). The spray capillary was then slid through a PEEK tee and sheathed by a piece of stainless steel (ss) HPLC tubing ( $1/16$  in. o.d., 200- $\mu\text{m}$  i.d., 75 mm long, Upchurch). The fused-silica capillary was cut to extend beyond the ss tubing by 3 mm and finally treated with hot sulfuric acid to remove the polyimide coating on the spray tip. A type K thermocouple (Heraeus, Hanau, Germany) was fixed on the outer surface of the ss tubing by a small piece of Teflon heat shrink tubing  $\sim 20$  mm from the front side of the ss tubing. A heating tape was then wrapped around

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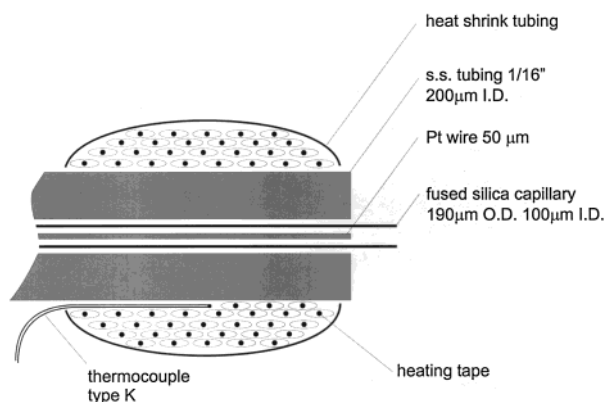


Figure 1. Detailed drawing of the electro spray ionization source with heated capillary (drawing not to scale).

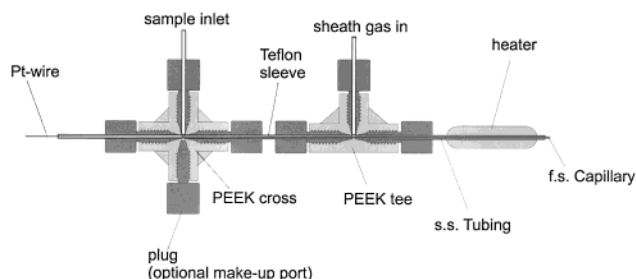


Figure 2. Assembly of the ESI ion source with spray capillary heater. For details of the heater, see Figure 1 (drawing not to scale).

the assembly (complete length of heated zone 45 mm) and fixed by heat shrink tubing.

The high-voltage (ESI voltage of the LCQ) was connected to the platinum wire by use of a clip lead. The ss tubing was grounded. The heating tape was coupled to a constant current voltage source which can be controlled manually. The thermocouple was linked to a digital thermometer.

The sample inlet was attached to the third port of the PEEK cross, while the fourth was reserved for optional makeup liquid (or similar use). The sheath gas outlet of the LCQ is connected to the PEEK tee. This is made not only to assist the spraying pneumatically but also to prevent droplets from getting into the grounded ss tubing and causing a short-circuit. The sheath gas pressure was adjusted to get an optimum analyte ion signal. The complete assembly was mounted on an acrylic plate and fixed on an XYZ positioner which could be attached in front of the external orifice of the mass spectrometer.

**Chemicals.** HPLC-grade dichloromethane (LabScan, Dublin, Ireland) and acetonitrile (Riedel-de Haën, Seelze, Germany) were dried over 3-Å molecular sieves by use of the dynamic method and used without further purification. Lithium trifluoromethanesulfonate was obtained from Aldrich (Deisenhofen, Germany).

The trityl dimer **1** was prepared just prior to analysis by adding 10 mg of zinc powder to a solution of 2.8 mg of chlorotriphenylmethane in 1 mL of acetonitrile. The suspension was shaken for 10 min and then filtered through a 0.2- $\mu$ m syringe filter (Teflon membrane, Alltech, Unterhaching, Germany). Mass spectrometry (chemical ionization with isobutane) proved complete absence of the chlorotriphenylmethane. The solution was used without further treatment.

Tetra(*p*-anisyl)hydrazine (**2**) was prepared according to a modified literature procedure<sup>16</sup> using standard Schlenk techniques: A solution of 200 mg of 4,4'-dimethoxydiphenylamine (Lancaster Synthesis, Mühlheim a.M., Germany) in 5 mL of dry acetone (Merck, Darmstadt, Germany) was cooled to 0 °C. A solution of 48 mg of potassium permanganate in 10 mL of dry acetone was added dropwise within 1 h. The reaction solution was allowed to stir for a further 4 h, until the color of the permanganate disappeared. The muddy brown suspension was then filtered through a glass frit, and the solvent was removed at 0 °C at reduced pressure. Recrystallization from dry benzene/pentane at 0 °C gave 114 mg of the product (57%) as slightly green colored crystals with a melting point of 84 °C.

**Procedures.** *Trityl Dimer 1.* A 10- $\mu$ L aliquot of the trityl dimer solution ( $\sim 5 \times 10^{-3}$  mol·L<sup>-1</sup>), obtained by treatment of chlorotriphenylmethane with zinc (vide supra), was diluted in 990  $\mu$ L of a dichloromethane/acetonitrile (50:50, v/v) mixture with addition of 1 mmol·L<sup>-1</sup> lithium trifluoromethanesulfonate to give  $5 \times 10^{-5}$  mol·L<sup>-1</sup>. The solution was infused at 2  $\mu$ L·min<sup>-1</sup> into the ion source. The temperature of the spray capillary-heater was first kept at room temperature and then increased in steps of 10–15 K to a final temperature of 140 °C. For each given temperature, at least 20 spectra were averaged and the signal intensity at *m/z* 243 was determined.

*Tetra(p-anisyl)hydrazine (3).* A 2.2-mg sample of **2** was dissolved in 1 mL of acetonitrile and then diluted 100-fold with a dichloromethane/acetonitrile (50:50, v/v) mixture with addition of 1 mmol·L<sup>-1</sup> lithium trifluoromethanesulfonate to give a concentration of  $5 \times 10^{-5}$  mol·L<sup>-1</sup>. While the solution was infused into the ion source at 2  $\mu$ L·min<sup>-1</sup>, the heater of the spray capillary was raised from room temperature to 140 °C in steps of 15–20 K. At least 20 spectra were averaged for each temperature to determine the signal intensity at *m/z* 446.

*Determination of the Maximum Spray Capillary Temperature.* The above solutions of the trityl dimer or tetra(*p*-anisyl)hydrazine (prepared as described above) were infused into the ion source of the mass spectrometer at 2  $\mu$ L·min<sup>-1</sup> while the capillary temperature of the ion source was raised from room temperature to 160 °C within 10 min. At the breakdown of the signal (disappearance of the signal, visible gas bubbles leaving the spray capillary), the indicated heater temperature was noted. The boiling point of the solution was determined using a Büchi 510 apparatus (Flawil, Switzerland). Boiling points are given as recorded without any corrections.

## RESULTS AND DISCUSSION

Figure 1 shows a schematic drawing of the design of our ion source, which is based on work done by Wang and Hackett.<sup>15</sup> It consists mainly of a fused-silica capillary, into which a platinum wire is inserted. High voltage is applied to this wire. The assembly is then slid through a grounded ss tubing, which is covered by a heating tape. The complete device acts as the spray capillary in the ESI process. By applying a voltage to the heating tape, the device could be heated to 200 °C. The temperature can be monitored by a thermocouple which is inserted between ss tubing and the tape. The authors are aware of opinions of other

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researchers<sup>17</sup> that the platinum wire does not need to be inserted into the fused-silica capillary that far, but for this special application it really needs to be, because the ionization by electrochemical oxidation<sup>13,14</sup> requires the surface of the electrode, which is the wire. So it needs to be put in that far, that it is almost completely within the heated region.

With this device, it is possible to acquire mass spectra of reaction solutions at elevated temperature. The upper temperature limit is given by the boiling point of the solvent used; i.e., it is restricted to  $\sim 150$  °C using common organic solvents. When the liquid is heated to temperatures higher than the boiling point, vaporization occurs and suppresses the ionization. At temperatures higher than the boiling point, no meaningful spectra could be acquired.

**Operation of the Ion Source.** For flow rates from 2 to 20  $\mu\text{L}\cdot\text{min}^{-1}$ , the spray tip was positioned to be 10–15 mm from the orifice of the mass spectrometer in an axial position. ESI voltages required for this mode of operation ranged from 1.5 to 3 kV, depending on what kind of solvent or analyte was used. For most analytes (e.g., peptides), the achieved signal intensity was at least 2 times better than with the conventional Finnigan electrospray source. For flow rates lower than 2  $\mu\text{L}\cdot\text{min}^{-1}$ , lower distances (down to 2 mm) from the spray tip to the orifice and even lower voltages (0.7–1.5 kV) were favorable because this resulted in a more stable spray and a better signal-to-noise ratio. It was possible to achieve stable spray conditions for flow rates as low as 0.1  $\mu\text{L}\cdot\text{min}^{-1}$ . For flow rates higher than 20  $\mu\text{L}\cdot\text{min}^{-1}$  and/or applications using nonvolatile buffers, it may be useful to move the spray tip into an off-axis position to prevent larger droplets or salt crystals from entering the mass spectrometer, but this application was not tested by the authors.

**Determination of the Maximum Spray Capillary Temperature.** The spray capillary is heated by the heating tape that surrounds it. At the same time, it gets cooled by several factors, i.e., heat transport to the nonheated end of the ss capillary, sheath gas flowing through the ss capillary and by the sample solution itself flowing through the spray capillary. For this reason, the temperature read by the thermocouple at the outer side of the ss capillary must be different from (i.e., higher than) the temperature of the analyte solution. So ways to correlate the “inner” and the “outer” temperature had to be found. When heating the heater to temperatures higher than  $\sim 140$  °C, we observed a signal breakdown, which could obviously be contributed to boiling of the analyte solution. At the same time, gas bubbles leaving the spray tip became visible. With this method, the heater temperature at which boiling of the analyte solution occurred ( $T_{\text{heater,max}}$ ) was determined. It was found to be  $144 \pm 3$  °C under the given operation conditions (solvent dichloromethane/acetonitrile, 50:50, v/v, with addition of 1  $\text{mmol}\cdot\text{L}^{-1}$  lithium trifluoromethanesulfonate, sheath gas flow, liquid flow rate, room temperature). The boiling point ( $T_{\text{bp}}$ ) of the solution (see above) was determined to be 59 °C. Room temperature ( $T_{\text{RT}}$ ) was 24 °C and all instrumentation was assumed to be initially at this temperature. So we made a simple linear correlation and got the following formula to calculate the temperature of the analyte solution ( $T$ ) for a given temperature of the heater ( $T_{\text{heater}}$ ).

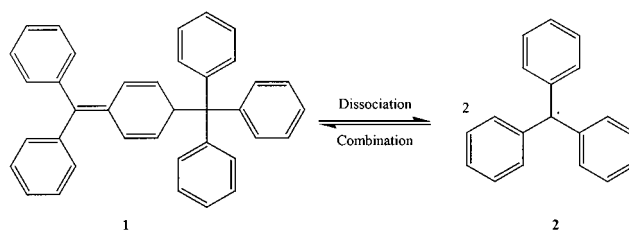
$$T = \frac{T_{\text{heater,max}} - T_{\text{bp}}}{T_{\text{heater,max}} - T_{\text{RT}}} T_{\text{RT}} + \frac{T_{\text{bp}} - T_{\text{RT}}}{T_{\text{heater,max}} - T_{\text{RT}}} T_{\text{heater}} \quad \{T_{\text{heater}} \parallel T_{\text{RT}} \leq T_{\text{heater}} \leq T_{\text{heater,max}}\} \quad (1)$$

And simplified for the measured temperatures:

$$T = 17 \text{ }^\circ\text{C} + 0.2917 T_{\text{heater}} \quad \{T_{\text{heater}} \parallel 24 \text{ }^\circ\text{C} \leq T_{\text{heater}} \leq 144 \text{ }^\circ\text{C}\} \quad (2)$$

All temperatures noted subsequently are determined this way.

**Thermal Dissociation of the Trityl Dimer.** The trityl dimer **1** and the trityl radical **2** in solution are in an equilibrium that is temperature dependent.



Under electrolysis conditions, using dichloromethane/acetonitrile (50:50, v/v) with addition of 1  $\text{mmol}\cdot\text{L}^{-1}$  lithium trifluoromethanesulfonate as the solvent, the ESI mass spectrum of **1** exhibits only one signal at  $m/z$  243 (Figure 3). The observed isotope pattern for this ion (isotopes separated by 1 Th) shows that this signal results from the oxidized radical **2**, rather than from a doubly charged trityl dimer **1**. It is assumed that the oxidation potential of the dimer **1** is too high to be suitable for ionization by electrochemical oxidation under the selected conditions.

The dimer **1** decomposes thermally; thus, the concentration of the radical **2** increases with increasing temperature. As no signal for the dimer is observed, its decrease in concentration cannot be monitored with this method. Figure 4 shows the measured temperature dependence of the signal intensity of the trityl radical **2** at  $m/z$  243. When comparing the measured intensities with trityl concentrations  $c_{\text{trityl}}$  that have been calculated using eq 3<sup>18</sup> ( $c_0$  being the initial concentration of the dimer **1**)

$$c_{\text{trityl}} = -\frac{e^{(T\Delta S_{\text{Diss}} - \Delta H_{\text{Diss}})/RT}}{4} + \sqrt{\frac{e^{2(T\Delta S_{\text{Diss}} - \Delta H_{\text{Diss}})/RT}}{16} + e^{(T\Delta S_{\text{Diss}} - \Delta H_{\text{Diss}})/RT} c_0} \quad (3)$$

and reported  $\Delta H_{\text{Diss}} = 44.8 \text{ kJ}\cdot\text{mol}^{-1}$  and  $\Delta S_{\text{Diss}} = 83.7 \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$  values,<sup>19</sup> good agreement up to 45 °C is found (see Figure 4).

**Thermal Dissociation of Tetra(*p*-anisyl)hydrazine.** Tetra(*p*-anisyl)hydrazine (**3**) decomposes thermally, giving two di(*p*-anisyl)nitrogen radicals **4**.

(18) The deduction of eq 3 is available as Supporting Information.

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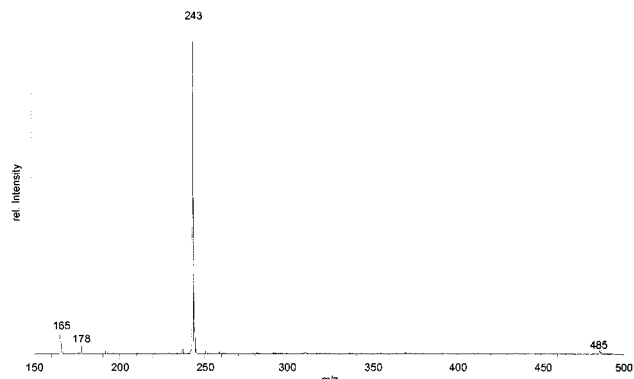


Figure 3. ESI mass spectrum of a solution of the trityl dimer **1** showing the signal of the oxidized trityl radical, solvent dichloromethane/acetonitrile (50:50, v/v) with addition of  $1 \text{ mmol}\cdot\text{L}^{-1}$  lithium trifluoromethanesulfonate, spray voltage  $2.3 \text{ kV}$ , flow rate  $2 \mu\text{L}\cdot\text{min}^{-1}$ .

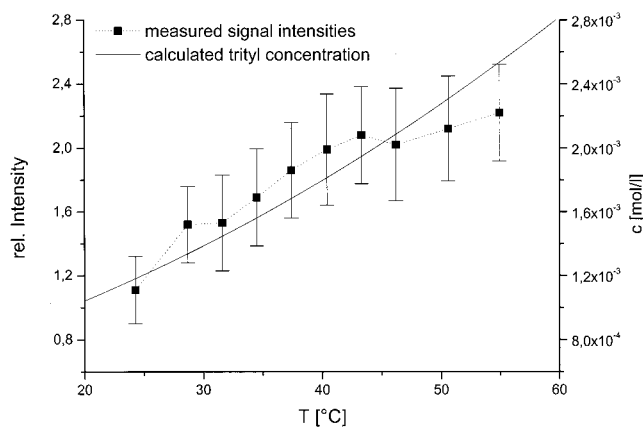
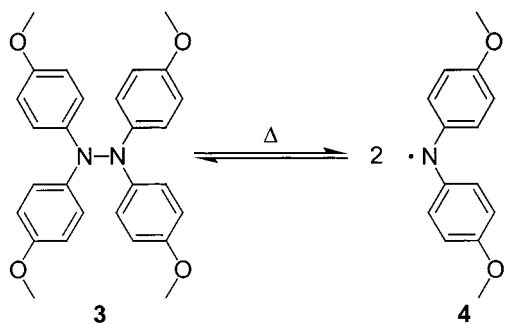


Figure 4. Temperature dependence of the signal of the trityl radical **2** at  $m/z$  243. Concentrations of **2** were calculated using eq 3.



The ESI mass spectrum of **3** includes a signal for the molecular ion at  $m/z$  456, proving a lower oxidation potential of **3** than of the trityl dimer **1**. The temperature dependence of the signal of **3** has been determined as noted above. Results are shown in Figure 5. The signal intensity decreases with increasing solution temperature, indicating the concentration decrease due to thermal

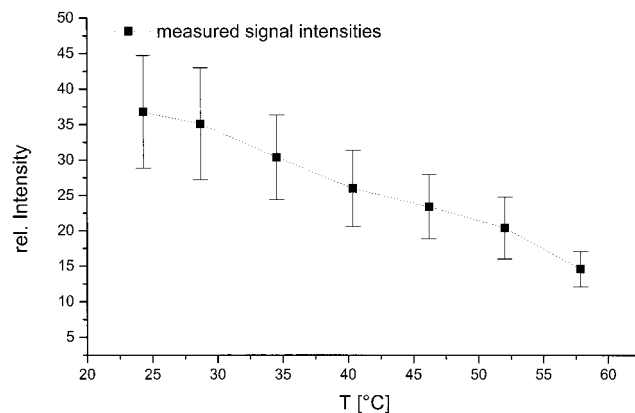


Figure 5. Temperature dependence of the molecular ion ( $m/z$  456) intensity of **3**.

decomposition. In this case, a comparison with calculated concentrations is not possible due to various reaction modes of the radicals and the resulting complex reaction kinetics.<sup>16,20,21</sup>

## CONCLUSIONS

The new type of ESI source has proven its capabilities to ionize thermally generated radical intermediates in reaction solutions using the ESI source as electrolytic cell. Methods to determine temperature dependence of compounds that are thermally unstable have been employed. With this new type of ionization source it becomes possible to investigate reactions in solution at elevated temperatures.

With the solvent used in these investigations, only a very small temperature range is accessible. Other solvents have been tested with this ion source and have been found to give almost the same results as with the conventional Finnigan ion source. So it should be possible to use the source with solvents that have a higher boiling point, thus enabling investigations of reactions at up to approximately  $150\text{--}200 \text{ }^\circ\text{C}$ . This will be the subject of future work.

## ACKNOWLEDGMENT

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## SUPPORTING INFORMATION AVAILABLE

Derivation of eq 3. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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