Wireless light-emitting device for the determination of chirality in real samples

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Abstract

Bipolar electrochemistry can be employed in the context of chiral recognition in order to obtain useful analytical readouts of enantiomeric analytes. Herein, we employ this concept for the simultaneous determination of two enantiomers present in solution, and its possible use as transduction mechanism for complex real matrices analysis. This approach is based on the combination of the enantioselective electrooxidation of only one of the two antipodes of a chiral analyte with the emission of light from light-emitting diodes (LEDs). A double hybrid device was designed, using the enantiomers of an inherently chiral oligomer and a bare gold wire as the anode and cathode of a green and red LED. By applying an appropriate voltage, the wirelessly induced redox reactions trigger light emission only when the probe with the right configuration is present in solution. This device was used to simultaneously measure the ratio between L- and D-Tryptophan, both present in solution and to quantify L-ascorbic acid in a commercial juice sample. The measurement correlates with the value reported on the sample specifications. These results illustrate the possible use of such light-emitting bipolar devices as analytical tools for qualitative and quantitative measurements of enantiomeric excess, even in real samples.

Keywords
Bipolar electrochemistry, chirality, light-emitting diodes, enantiomeric excess, real sample analysis.

1. Introduction

Amino acids are one of the most important types of natural compounds due to their involvement in essential and well-known physiological processes. For example, in biology, commonly the L-form of amino acids largely dominates, thus, high levels of certain D-amino acids have been attributed to bacterial activity in food or to different pathologies, such as chronic kidney disease and neurological disorders, like schizophrenia and Alzheimer's disease. Therefore, simultaneous monitoring and quantification of D- and L-amino acids for biomedical applications, as a marker of quality, authenticity and food safety, is highly needed [1]. Commonly, simultaneous detection of both enantiomers of amino acids has been carried out mainly through high-performance liquid chromatography, which requires time-consuming protocols and expensive instrumentation. In addition, alternative procedures such as capillary electrophoresis [2], reactive extraction [3], or ultrafiltration through enantioselective membranes have been developed [4]. Recently a different approach to distinguish the enantiomers of two classes of amino acids in blood and plasma has been proposed, involving "on the fly" bio-detection [5]. However, this type of detection was only possible by analyzing the enantiomers in separate solutions, limiting the analysis of mixtures, often present in real samples. An interesting alternative is the development of fast and straightforward optical read-outs of different electroactive analytes [6-9]. Recently electrochemistry-based light-emitting systems have gained considerable attention due to the possible direct visualization of chemical information. Different light-emitting systems, based

on electroluminescence [10-14] or fluorescence [15] have been exploited for the quantification of different analytes of interest [16-20]. However, these methods require the use of additional chemicals, such as luminophores, co-reactants, fluorescent dyes, and often the light emission is triggered by complex reactions pathways. A promising alternative is the use of light-emitting diodes (LEDs) for the direct visualization of electrochemical processes. In such devices, the analytical information is directly encoded by the electric current passing through the LED and the concomitant light emission. Although light emission can be triggered by a direct electrical connection [21], an interesting approach is to power these devices in a wireless manner, via coupling thermodynamically spontaneous reactions to the terminals of the LED [22,23] or by using bipolar electrochemistry (BPE) [24,25]. The wireless nature of the BPE approach has been proven to be very convenient for materials science and analytical applications [26-32]. Recently BPE has been used for the electroanalytical determination of chiral information coupled with mechanical actuation or light emission [33-35]. These systems are based on the enantioselective oxidation of a chiral probe on the surface of an inherently chiral oligomer [36], coupled with the reduction of a pristine polypyrrole strip, leading to a selective electron-flow. The selectivity of these systems is based on the diastereomeric interactions between the inherently chiral oligomer and the antipodes dissolved in solution. Therefore, the actuation of the polypyrrole strip or the light emission occurs only in the presence of the right enantiomer. Herein, we take advantage of such a highly enantioselective light emission to design a double light-emitting bipolar recognition device for the simultaneous analysis of enantiomeric mixtures of amino acids in real samples. The light emitting bipolar device is designed by combining three main ingredients; (i) an inherently chiral oligomer, which is the site of enantioselective oxidation; (ii) pristine gold wires, where the reduction reaction occurs; and (iii) a micro-LED for the

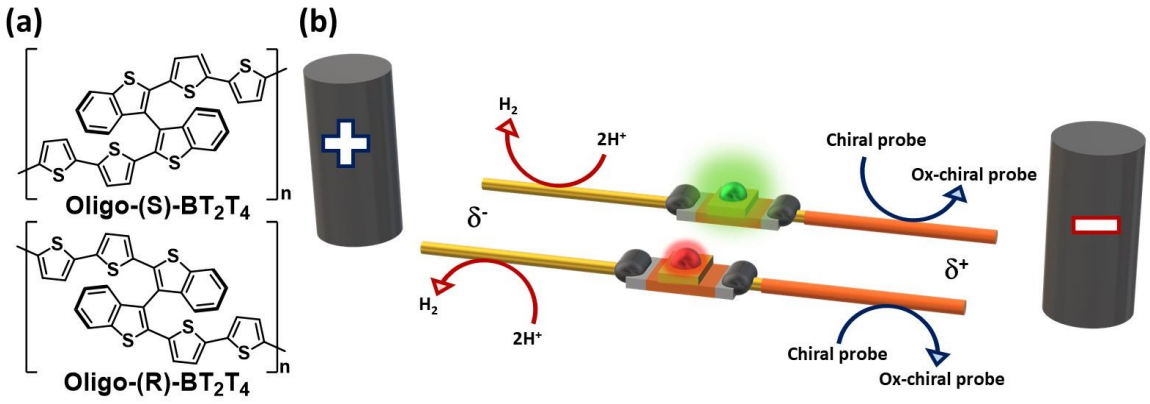
light emission (Scheme 1). This hybrid device is placed in a solution containing enantiomers of two different chiral model analytes; Tryptophan (Tryp) and Ascorbic Acid (AA). Tryp is an essential amino acid used commonly to balance the nitrogen content in adults and also involved in the growth of infants [37]. Tryp antipodes were chosen to be simultaneously detected and quantified by using the double LEDs bipolar device. Finally, AA was chosen for its high abundance in fresh fruit juice, especially blackcurrants, citrus fruit and strawberries. In addition, L-ascorbic acid is one of the most important water-soluble vitamins, since it acts as a free-radical scavenger of reactive oxygen and nitrogen species [38,39]. Due to its relatively low oxidation potential, different classic electrochemical methods have been used for ascorbic acid quantification in complex media [40]. However, a straightforward visual readout of the enantiorecognition of such a chiral probe has not been explored so far.

2. Experimental

$LiClO_4$ (Aldrich, 99%), anhydrous acetonitrile (Aldrich, 99.9%), Au wires (d = 0.25 mm, Alfa Aesar, 99.9%), L-ascorbic acid (ACS reagent, ≥ 99%), L- and D-Tryptophan (Aldrich, ≥ 99%), agar silver paint (Agar scientific Ltd.) and miniaturized red (0603 SMD diode, Wurth Elektronik, 1.60 mm × 0.8 mm) and green light-emitting diodes (LED, 0603 SMD diode, OSRAM Opto Semiconductors, 1.70 mm × 0.8 mm) (Table S1) were used as received. Enantiopure (*R*)- or (*S*)-2,2′-bis(2-(5,2′-bithienyl))-3,3′-bithianaphthene ((*R*)-BT_2T_4 and (*S*)-BT_2T_4, scheme 1a) were synthesized following a published methodology [36]. All solutions were prepared with deionized water (MilliQ Direct-Q®).

Enantiopure oligo-(*R*) and oligo-(*S*)-BT_2T_4 films were obtained according to previous reports [33-35]. Electrodeposition was carried out in a 0.1 M $LiClO_4$/ACN solution, containing 1

mM of monomer by chronopotentiometry applying 1.5 mA for 25 minutes. A gold wire was used as working electrode together with a platinum mesh and Ag/AgCl as counter and reference electrodes, respectively. The length of the Au wire modified with the correspondent oligo-(*S*)- or oligo-(*R*)-BT_2T_4 is approximatively 0.75 cm. The double bipolar devices were assembled by connecting a wire modified with the corresponding oligo-BT_2T_4 film and an unmodified Au wire to the anode and the cathode of the LEDs, respectively (Scheme 1b). In brief, the Au electrodes with the desired dimensions and the LED were attached to a glass support using double-sided adhesive tape. A small volume of agar silver paint was employed to establish an electric connection between the electrodes and the LED. The total length of the final bipolar electrode is 1.8 cm and the spacing between the parallel bipolar electrodes is 1 cm. For the bipolar recognition, the enantiopure oligo-(*R*)- and oligo-(*S*)-BT_2T_4-LED-Au devices were fixed in parallel at the center of a bipolar cell. Two graphite feeder electrodes were positioned at the extremities of the cell at a distance of 5 cm. The first recognition experiments were carried out in an aqueous 5 mM $LiClO_4$ solution in the presence of different ratios of the Tryp enantiomers (90:10 and 10:90) by varying the electric field from 0 V cm^{-1} to 3.6 V cm^{-1}. The experiments with different concentrations of L-AA (2, 4, 6, 8 and 10 mM) were carried out at a constant electric field (2 V cm^{-1}). The standard addition method (from 2 to 10 mM) was used to carry out the quantification of L-AA in a commercial lemon juice sample, at a constant electric field (2 V cm^{-1}). Experiments were monitored by using a CCD camera (CANON EOS 70D, Objective Canon Macro Lens 100 mm 1:2.8). Images were processed with Image J software.



Scheme 1. (a) Chemical structures of the two enantiomers of BT_2T_4 oligomers. (b) Schematic illustration of the bipolar set-up used for the wireless quantification of chiral probes based on the double light emission with a representation of the associated chemical reactions. The orange part stands for the BT_2T_4 oligomer. The distance between the feeder electrodes is 5 cm and the length of the bipolar object is 1.8 cm.

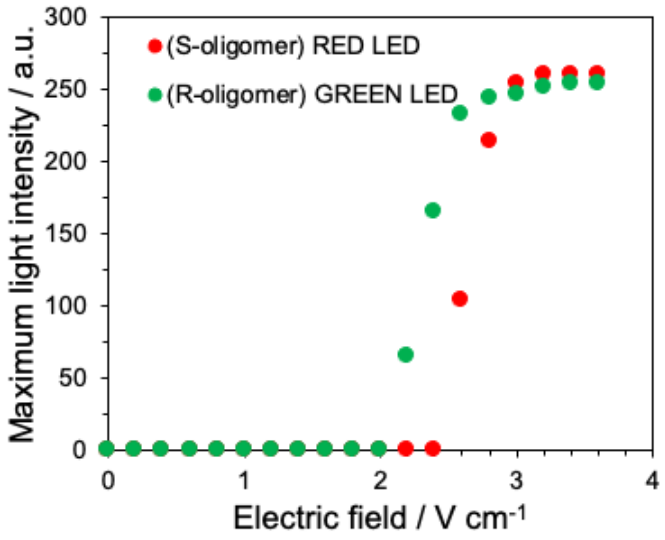
3. Results and Discussion

In order to evaluate the electric field required to switch on the LED integrated in each bipolar electrode, a non-enantioselective green and red Au-LED-Au system was tested first. The double LED device was placed at the center of a bipolar cell, containing an aqueous 5 mM $LiClO_4$ solution. To induce redox reactions in such a set-up, an electric field, high enough to sufficiently polarize the bipolar electrode at its extremities, was applied. Under these conditions, the oxidation and reduction of water at the anode and cathode of the LED, respectively, trigger the light emission of the diode. At low electric field values (below 2 V cm^{-1}), there is not enough driving force to induce these redox reactions, thus both LEDs remain in the off-state (Figure S1). Once the electric field reaches a threshold value (around 2 and 2.4 V cm^{-1} for the green and red LED, respectively) the polarization potential

difference, with respect to the surrounding solution, induces the oxidation and reduction of water at the extremities of both Au-LED-Au bipolar electrodes. This creates an electron flow from the anodic to the cathodic side of the LED and thus a concomitant light emission. As it can be seen from Figure S1, the emitted light intensity is a function of the electric field value. However, the difference of threshold value (0.4 V cm^{-1}) between the green and red LED is attributed to the intrinsic resistance of the diode.

After the evaluation of the threshold potential of a non-enantioselective set up, the influence of the inherently chiral oligomer on the threshold potential of the same system, in the absence of a chiral probe, was studied. At first, a device combining a red oligo-(*S*)-BT_2T_4-LED-Au and green oligo-(*R*)-BT_2T_4-LED-Au system was placed at the center of a bipolar cell containing only an aqueous 5 mM $LiClO_4$ solution. In the absence of the chiral probe, water oxidation and reduction remain the redox reactions occurring at the extremities of the bipolar electrode. Thus, a similar behavior of the light intensity as a function of the applied electric field was observed for the double functionalized device (Figure 1) in comparison with the non-enantioselective system (Figure S1).

Below 2 V cm^{-1} both LEDs remains switched-off, whereas, around 2 and 2.4 V cm^{-1} (for the green and red LED, respectively) the electric field reaches the threshold value inducing the light emission. An identical response was observed for the inverted configuration, combining a red oligo-(*R*)-BT_2T_4-LED-Au and a green oligo-(*S*)-BT_2T_4-LED-Au (Figure S2), with a similar difference of threshold potential (0.4 V cm^{-1}) between the green and red device. Thus, in the presence of the inherently chiral oligomers and the absence of a chiral probe, the threshold potential is governed by the intrinsic resistance of the LED.



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158 Figure 1. Maximum light intensity as a function of the applied electric field for the
159 combination of a red oligo-(*S*)-BT_2T_4-LED-Au and green oligo-(*R*)-BT_2T_4-LED-Au system
160 in an aqueous solution of 5 mM $LiClO_4$.

161 The first step, before testing the device in the presence of a chiral probe, was to verify by
162 differential pulse voltammetry (DPV) the enantioselectivity of (*R*)- and (*S*)-BT_2T_4 oligomers
163 deposited on a gold wire with respect to the enantiomers of Tryptophan, L-Tryp and D-Tryp,
164 in separate solutions (4.5 mM). The DPV shows a peak-to-peak separation of approximately
165 700 mV on (*S*)-oligomer modified electrodes for the electrooxidation of the Tryp enantiomers
166 (Figure S3). This very high thermodynamic differentiation allows the simultaneous detection
167 of both Tryp enantiomers with a double light-emitting device. The red oligo-(*S*) and green
168 oligo-(*R*)-BT_2T_4-LED-Au system was placed in the center of a bipolar cell containing an
169 aqueous 0.5 mM $LiClO_4$ solution. Theoretically, in the presence of both chiral probes, by
170 varying the electric field, both LEDs will switch on, and their light intensity should correlate

with the concentration of the corresponding enantiomer. For these measurements, a ratio of 90:10 D:L-Tryp (4.5, 0.5 mM, respectively), and the specular one 10:90, have been chosen.

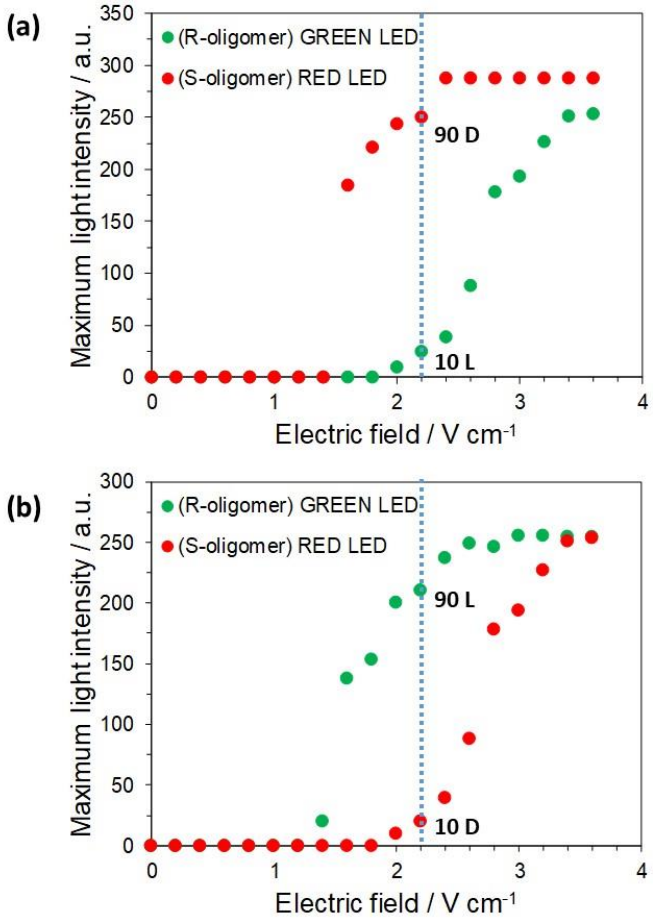


Figure 2. Maximum light intensity as a function of the applied electric field for a red oligo-(*S*)-BT_2T_4-LED-Au and green oligo-(*R*)-BT_2T_4-LED-Au system in (a) an aqueous solution of 5 mM $LiClO_4$ containing 4.5 mM D-Tryp and 0.5 mM L-Tryp and (b) an aqueous solution of 5 mM $LiClO_4$ containing 0.5 mM D-Tryp and 4.5 mM L-Tryp.

Under these conditions, the light intensity for the oligo-(*S*)-BT_2T_4-LED-Au device is higher than the one related to the oligo-(*R*)- BT_2T_4-LED-Au when the double recognition device is

placed in the 90 D : 10 L solution (Figure 2a). The specular response was obtained by placing the same device in the 10 D : 90 L solution (Figure 2b). This is coherent with the DPV measurements (Figure S3) showing a preferential oxidation of D-Tryp on the (*S*)-oligomer modified wire. When comparing the light intensity for the oxidation of D- (250 a.u.) and L-Tryp (25 a.u.) at 2.2 V cm^{-1}, the values correlate with the 90:10 D-Tryp/L-Tryp ratio present in solution. Similar results were obtained with the opposite ratio, D- (10 a.u.) and L-Tryp (210 a.u.) at 2.2 V cm^{-1},

In the next step, the threshold potential of the device in the presence of L-AA was evaluated. The diastereomeric interaction between the (*S*)-oligomer and L-AA is more favorable from a thermodynamic point of view. Differential pulse voltammograms show a peak-to-peak separation of approximately 100 mV for the electrooxidation of L-AA on (*S*)- and the (*R*)-oligomer modified electrodes, respectively (Figure S4). Although this peak separation is smaller in comparison with those observed for other chiral probes (e.g. 600 mV for DOPA enantiomers) [33-35], it is still high enough for bipolar recognition experiments. Since for this set of experiments the enantiomeric probe was available in only one configuration, the double device was built with only the green LEDs that are the ones having a lower intrinsic resistance (Figure S5a). The green oligo-(*S*) and oligo-(*R*)-BT_2T_4-LED-Au system was placed at the center of a bipolar cell containing an aqueous 5 mM $LiClO_4$ solution in the presence of 10 mM L-AA. Under these conditions, the threshold potential for generating light at the (*S*) bipolar electrode is lower (1.4 V cm^{-1}), than the one needed for the (*R*) device to switch on (1.8 V cm^{-1}), thus allowing chiral discrimination. An analog light emission behavior was observed when the red oligo-(*S*) and oligo-(*R*)-BT_2T_4-LED-Au system was

used (Figure S5b), with a similar difference in threshold values (0.4 V cm^{-1}) between the enantiopure electrodes.

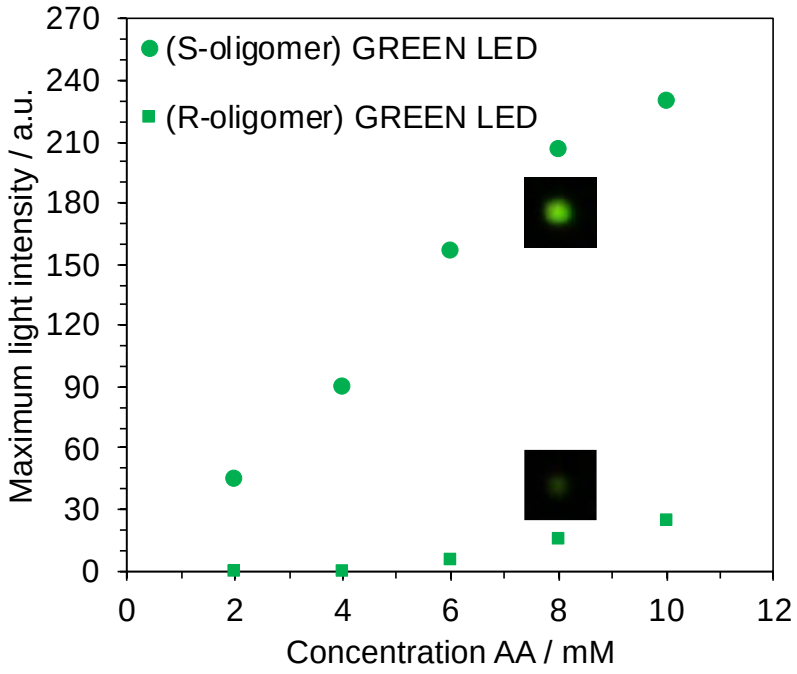


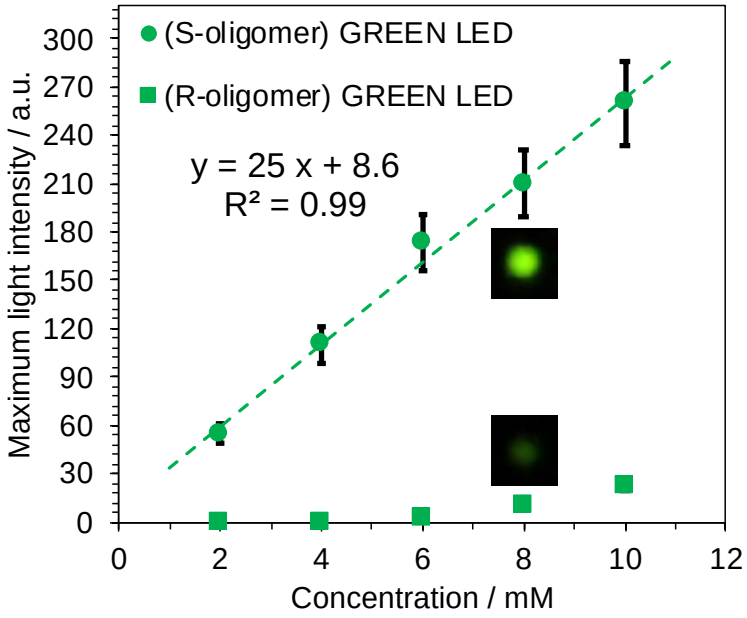
Figure 3. Maximum light emission as a function of L-AA concentration measured by the enantioselective oxidation of L-AA on a double green oligo-(*R*)- and oligo-(*S*)-BT_2T_4-LED-Au bipolar device, in a 5 mM $LiClO_4$ solution at a constant applied electric field (2 V cm^{-1}). Inset: optical pictures of the (*S*)- and (*R*)-BT_2T_4-LED-Au bipolar device at a given L-AA concentration of 8 mM.

After demonstrating chiral discrimination through an on-off light emission, the next step was to evaluate the possibility of using the concept for the quantitative analysis of L-AA. The light intensity was measured as a function of the enantiomer concentration. Five L-AA samples were analyzed (2, 4, 6, 8 mM and 10 mM) with a green oligo-(*R*)- and oligo-(*S*)-BT_2T_4-LED-Au bipolar device by applying a constant electric field (2 V cm^{-1}). For each

concentration, the average light intensity over a period of 10 sec has been used, in order to eliminate small fluctuations of the light intensity during the experiment caused by the fast consumption of the analyte. A plot of the light intensity as a function of L-AA concentration reveals a linear correlation (R^2 = 0.983) for the oligo-(*S*)-BT_2T_4-LED-Au with a high sensitivity ($m_{(S)}$ = 24.3 a.u./mM), in contrast to the oligo-(*R*)-BT_2T_4-LED-Au, which shows, as expected, almost no signal (Figure 3). This sensitivity and linearity are comparable to the ones obtained with classic electrochemical systems that require sophisticated electrode materials [41,42]. This illustrates the possible use of such a light-emitting bipolar device as an analytical tool for the qualitative and quantitative measurement of enantiomers with relatively close oxidation potential.

Finally, to test the reliability of the results in real samples, the quantification of L-AA in commercial lemon juice was performed. Recently, different bipolar electrochemical light-emitting systems, based on electrochemiluminescence, have been developed for the quantification of AA in aqueous solution [43,44]. However, the light response is based on indirect electrochemical mechanisms, without the possibility to directly distinguish between the enantiomers of the analyte.

Before starting the experiments, the sample had to be diluted (12.5%) due to the turbidity of the sample, which might interfere with the light response. First of all, light emission of the diluted juice was measured using a green oligo-(*S*)- and oligo-(*R*)-BT_2T_4-LED-Au bipolar device, at a constant electric field (2 V cm^{-1}). Results show, as expected, enantioselective on-off recognition of L-AA, with the green LED switching on only if connected to the (*S*)-enantiomer of the oligomer (Figure 4).



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238 Figure 4. Maximum light emission as a function of L-AA concentration measured by the
239 enantioselective oxidation of L-AA on a double green oligo-(*R*)- and oligo-(*S*)-BT_2T_4-LED-
240 Au bipolar device, in 12.5% commercial lemon juice at a constant electric field (2 V cm^{-1}).
241 Error bars refer to measurements carried out three times on the same bipolar device. Inset:
242 optical pictures of the (*S*)- and (*R*)-BT_2T_4-LED-Au bipolar device at a given L-AA
243 concentration.

244 The standard addition method was selected for the quantification of L-AA in commercial
245 lemon juice. Five solutions with different standard concentrations, from 2 to 10 mM, were
246 prepared. Plotting the maximum light intensity as a function of concentration leads to a linear
247 correlation (R^2 = 0.99) for the oligo-(*S*)-BT_2T_4-LED-Au, and again hardly any emission is
248 recorded for the oligo-(*R*)-BT_2T_4-LED-Au (Figure 4). By using the regression equation y =
249 25x + 8.6, with y being the light intensity and x the concentration, a L-AA concentration of

2.7 mmol L^{-1} was calculated, which is in very good agreement with the L-AA content reported in the lemon juice specifications and in the literature (2.5 mmol L^{-1}) [45].

4. Conclusions

A bipolar electrochemical device, modified with inherently chiral oligomers has been designed for the simultaneous optical determination of the enantiomers of Tryptophan, present in solution, and the quantification of L-ascorbic acid in real samples. The concept of wireless light emission was successfully used for the determination of the enantiomeric ratio between the two configurations of Tryp, both present in the same solution. The average light intensity was found to be directly proportional to the analyte concentration and used for the direct quantification of L-ascorbic acid in commercial lemon juice through the standard addition method. The calculated values are in perfect agreement with those found in the literature and with the ones indicated on the lemon juice specifications. The sensitivity and linearity achieved for L-AA quantification are comparable to those obtained with classic electrochemical systems, that require sophisticated electrode materials. The presented approach can thus be considered as an efficient and complementary alternative for chiral discrimination with respect to the common spectroscopic methods. Moreover, since the same device has been employed for all the experiments presented herein, it can be used for multiple determinations without noticeable fouling effects which might interfere with the measure of light emission. These results open up the possibility to use such double light-emitting bipolar devices as straightforward analytical tools for the qualitative and quantitative discrimination of enantiomers, even when they have relatively close oxidation potentials on a given chiral selector layer.

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