Rotating droplet as a new alternative for small volume electrochemical measurements

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Abstract

We present a simplified and flexible implementation of the rotating droplet (RD) method as an alternative to rotating disk electrode for electrochemical measurements in a small volume system. The rotating droplet system is based on the principle of a rotating disc electrode, but the method uses only single droplet of investigated solution to perform analysis. In our system the disk and the ring electrodes of a traditional rotating ring disk electrode are used as reference and counter electrodes, respectively. This makes it possible to use any flat, conducting surface as a working electrode. We also show that the RD method can be useful for fast detection of analytical samples present in the gaseous atmosphere around the droplet.

1. Introduction

Kinetic methods can be used to monitor the progress of a chemical reaction, crucial for describing the mechanism of reactions and determination of factors which influence them. In the case of rapid reactions very fast detection methods are needed and, to overcome the problem of slow mass transport, hydrodynamic methods may be used either in a configuration where the liquid is flowing with respect to a static working electrode, or when an electrode induces liquid movement through its own motion [1–3]. Both approaches lead to the same result: mass transport is controlled by the experimental settings, resulting in elimination of limitations of the signal caused by slow diffusion. Monitoring reactions with electroactive species has historically been done using a rotating disk electrode (RDE) [4,5], for which the liquid movement principles are well described analytically. Notwithstanding, usage of RDE systems is associated with several problems such as the necessity of utilizing large sample volumes (at least millilitres),

and severe limitations on the available working electrodes. Microfluidic systems are also often used, and their behaviour and electrochemical response, dependent on mass transport in the channel, is also known. However, the usage of microfluidic system also has some limitations; the preparation of a channel with specialized electrodes can be both time-consuming and bothersome, and, also here, a relatively large sample volume is needed (even if a microchannel is used one has to remember filling syringes, connection tubes etc.). Despite the many advantages of known systems, new methods characterized by simpler preparation and less volume needed for analysis are desired. In 1997 Gratzl et al. proposed a method where a small droplet deposited on a flat surface was exposed to a mild jet of humidified nitrogen which caused it to rotate [6]. Another approach was made by Kurmitz et al. who showed rotation of a droplet with the use of a rotating disc electrode tip. In this system, used for determination soil enzyme activities, the reference and auxiliary electrodes were immersed into the rotating volume. However, immersion of wires into the rotating 40 µl droplet could induce turbulent flow which is much more difficult to represent mathematically [7,8]. In 2014 Noel et al. used a three-electrode system with all of the electrodes on a screen printed setup at the bottom of the droplet [9]. They reported this as a well-defined method for effective mixing of the sample solution which allows rapid detection of analytes and they showed that the response of the RD system is described by the Levich equation used for the regular RDE setup, but with a different initial constant.

Here we present a method which combines the advantages of RDE by improving upon the previously proposed RD methods. However, it also exceeds those in simplicity of construction. Our RD method uses a rotating ring disc electrode to create the rotation of the droplet. This also serves as a base for the electrochemical system, where the platinum ring plays the role of counter electrode, and the disc, covered with electrodeposited silver, is used as the pseudo-reference electrode (see scheme in Fig 1). This design allows us to keep a constant potential over the experimental time. Both auxiliary and reference electrodes are placed on the rotating tip, whereas working electrode is placed at the bottom of the droplet. Therefore, there are no limitations on the structure and coverage of the working electrode and any conducting material can be used to that purpose.
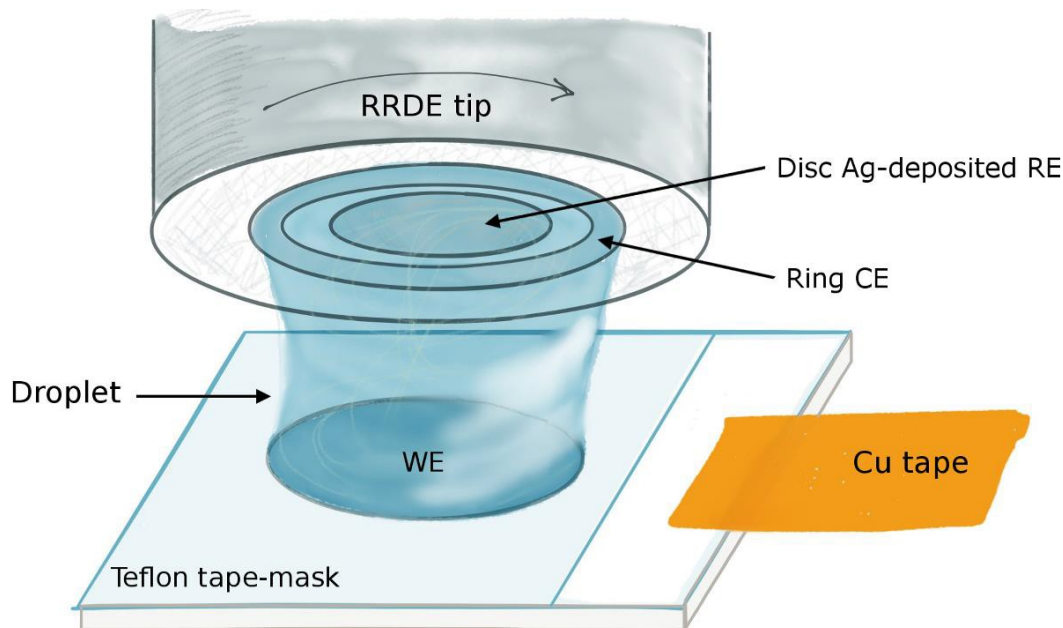
Figure 1 Schematic illustration of the experimental setup

In contrast to the handful materials used for RDE analysis the new RD method allows us to use any working electrode material, including materials adsorbed on the surface of electrode. Carbon nanoparticles, known as a material of extremely high surface area, good electrical conductivity, and many reactive surface sites, which can help performing reaction with higher efficiency, have frequently been used for electrode layers. In this article the enzymatically catalysed oxygen reduction reaction has been chosen as a model It is a well-known and environmentally important reaction. In our research we have used Bilirubin oxidase (BOx) from *Myrothecium verrucaria* as a catalyst for ORR [10]. The deposition of both supporting material and catalysts at the electrode surface has already been obtained in many ways, e.g. by physical adsorption [11], interaction with polyelectrolytes, by layer by layer methods [12,13], and encapsulation in sol-gels [14], as was used in our study. We also show that the RD system allows for very quick response to a change of the atmosphere around the droplet.

2. **Experimental section**

2.1.Chemicals and materials

KNO$_3$, AgNO$_3$, Na$_2$HPO$_4$ and NaOH were purchased from POCh. Negatively charged carbon nanoparticles (CNP) were from Cabot Corporation. N-Trimethoxysilylpropyl-N,N,N-trimethylammonium chloride (50%) in methanol (TMA) was purchased from ABCR.

Tetramethoxysilane (TMOS) was from Sigma-Aldrich, methanol, ethanol, acetone, isopropanol, and HCl were obtained from Chempur.

Indium tin oxide (ITO) coated glass plates (resistivity 8–12 Ω sq^{-1}) were purchased from Delta Technologies, Ltd., USA. BOx was donated by Amano Enzymes Inc. Water was filtered and demineralized using an ELIX system (Millipore). The Rotating Disc Electrode (RDE) system, and rotating ring-disc electrode tips were from ALS.

2.2 Fabrication of the system

ITO slides (25 x 50 x 1.1 mm) used as a working electrode support were cleaned with acetone, isopropanol, ethanol, then with deionized water and heated for 10 min in a tube furnace at 500 °C in air. The area of the (WE) was masked and limited by hydrophobic tape. The working electrode surface was bare ITO or ITO modified by the CNP-BOx solgel prepared by mixing CNPs and enzyme with a TMA solution prepared as shown previously [15]. Platinum RRDE was used as counter and reference electrodes. As a counter electrode the platinum ring was used. The reference electrode was constructed by electrochemical modification of the platinum disc from the RRDE, performed with cyclic voltammetry scanning in 20 mM solution of AgNO$_3$ in water, 30 scans from -0.1 to 0.1 V, obtaining a silver layer on the top of rotating disc. The ITO plate with masked area placed in the bottom whereas RRDE tip placed in the top. Using a micro-pipette the electrolyte solution with the electroactive analyte was gently injected between the electrodes and surface tension keeps it in a droplet shape. Rotating the upper disc electrode causes the whole droplet volume to start spinning.

The electrochemical experiments were performed using an Autolab PGSTAT30 (Metrohm Autolab) electrochemical system.

3. Results and Discussion

3.1 Characterisation of the Rotating Droplet system

The system described above was subject to systematic characterization and compared with the standard RDE method. In case of RD, the droplet of electroactive solution was deposited on a bare ITO surface masked with hydrophobic tape and sandwiched with the rotating disc tip at a given distance. In order to evaluate the impact of rotation velocity, the sensitivity for the height of the droplet has been measured, shown in figure 1. A. and B. To this end we measured the

reduction of 0.1 mM solution of $K_3Fe(CN)_6$ in 0.5 M KNO_3 using cyclic voltammetry (scan rate 20 mV s^{-1}, potential range -0.2 to 0.6 V) with different heights of the droplet and at two different rotation rates.
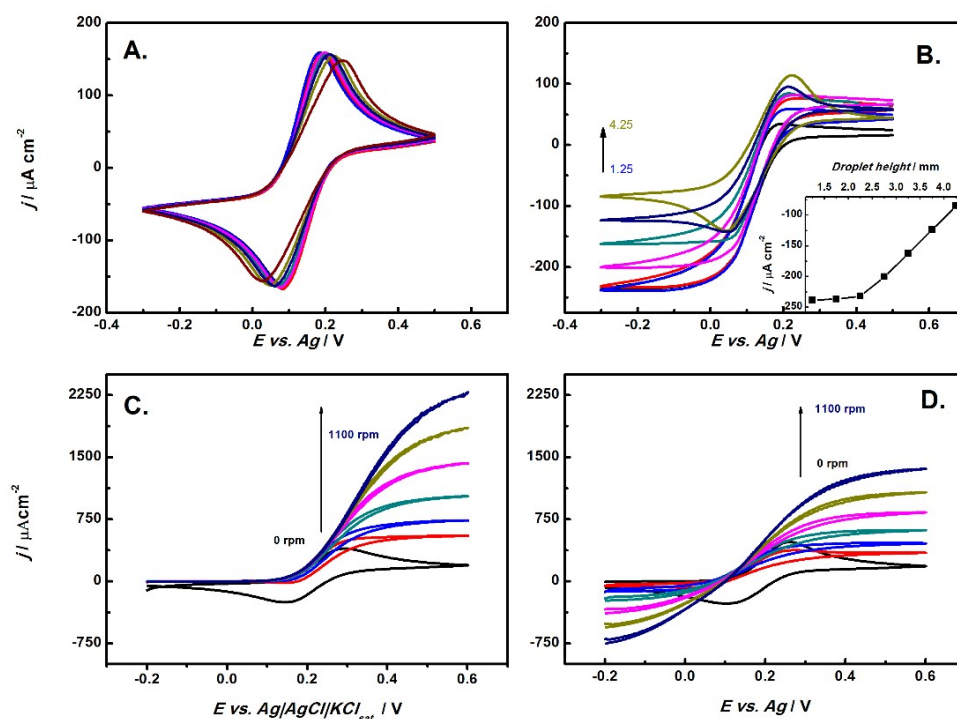

*Figure 2 Cyclic Voltammograms of 0.1 mM $K_3Fe(CN)_6$ in 0.5 M KNO_3 at **A.** 0 rpm and **B.** 500 rpm in RD system at different heights of the droplet (in millimeters) and cyclic voltammograms 0.1 mM $K_4Fe(CN)_6$ in 0.5 M KNO_3 in **C.** RDE system **D.** RD system under different rotation rates, scan rate 20 mV s^{-1}.*

For static conditions almost no difference between the obtained "duck"-shaped voltammograms in range of 1,25 to 4,75 mm of the droplet height is visible. In contrast, the hydrodynamic signals differ from each other depending on the droplet height. This behaviour is caused by the damping of the liquid movement in the bottom part of the droplet. The cyclic voltammograms change from convection-limited to peak-shaped diffusion limited as the height is increased. It is worth noting that for a height up to ca 2.25 mm the current is independent of the droplet height, in agreement with the findings by Noel et al.. As a compromise between effectiveness and ease of use a 2.75 mm droplet height was used in the further experiments. Figure 1 C and D show the cyclic voltammograms obtained for oxidation of the redox probe $K_4Fe(CN)_6$ in 0.1 mM solution of electrolyte 0.5 M KNO_3 in RDE and RD system, with different rotation rates

from 0 to 1100 revolutions per minute (rpm). RDE measurements were performed on a standard GCE tip, with Ag/AgCl as a reference and Platinum wire as a counter electrode. The RD system uses bare ITO as a working electrode and reference and counter as described earlier.

The responses obtained for the standard RDE system shows the expected sigmoidal shaped voltammograms. Similarly, using the RD method we can control mass transfer rate by rotating rate and as a result a sigmoidal shape is obtained. The RD results still follow the same behaviour described by the Levich equation but with a different initial constant (0.325 instead of 0.620) reflecting the lower liquid speed at the bottom of the droplet. The cathodic current in the RD measurements is caused by reduction of the product from the oxidation reaction. **In normal RDE experiments the product of the reaction is diluted into the large sample volume and is not detected. In this case, the volume is small and well mixed, so the reduced $Fe(CN)_6^{3-}$ can be oxidised back to $Fe(CN)_6^{4-}$.**

3.2 Electrochemical response from oxygen reduction

RD shows great utility for semiconducting electrodes covered with carbon nanoparticles and protein films for enzymatic reactions. Here the oxygen reduction reaction in RD is strongly dependent on the rotation rate. In this article we present recent studies on a system which uses the open boundaries of the droplet, which enable the fast diffusion of gasses through the droplet boundaries, to its advantage.
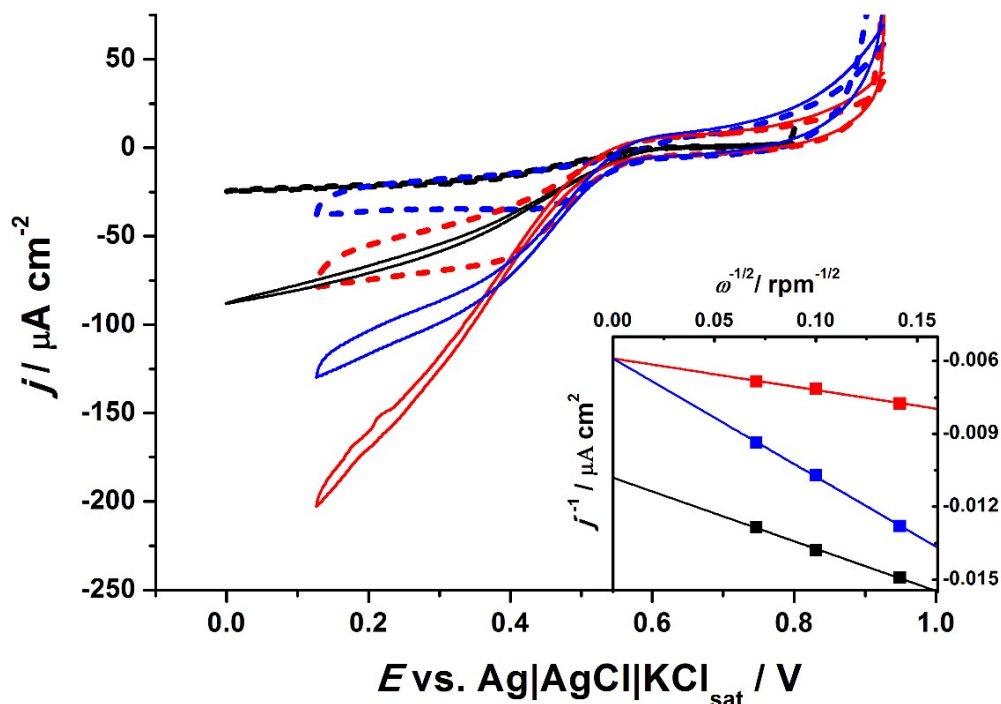
Figure 3 Cyclic voltammograms in 0.1 mM PBS buffer pH 4.8 in air saturated solution on ITO electrode (blue curve), air saturated solution on glassy carbon RDE (black curve) and oxygenated solution on ITO electrode (red) with different rotation rates Dashed lines - 0rpm, full lines - 200 rpm. Insert Koutecky-Levich plot for air and oxygenated solutions on RD and aerated solution on RDE. Scan rate 2 mV s-1, j taken at 0.2 V.

Fig.2 shows difference of performance in solution containing oxygen of the standard GCE RDE and an ITO electrode in the new RD system. Both electrodes were covered with CNPs and BOx encapsulated in a TMA sol-gel matrix. That matrix, compared to previously used matrices based on TMOS or MTMOS [14] shows a greater stability under hydrodynamic conditions. The RD consistently exhibit higher efficiency towards oxygen reduction than the RDE, most likely due to better compatibility of the sol-gel matrix with ITO than with glassy carbon, something we have observed previously.

For further analysis, chronoamperometric measurements were performed. Here we present the fast response from the system to a change of the atmosphere around the droplet. In fig 3A we see the how quickly the current changes to the changing atmosphere. The first response of the system is visible within 1 s. and 93 % of the total signal was observed after 50 s. A comparison between the response time during rotation and when the drop is stationary can be seen in Fig 3B. At the beginning humidified inert Argon was applied for 200 seconds, later the gas source was exchanged to oxygen, after 400 s argon was applied anew.
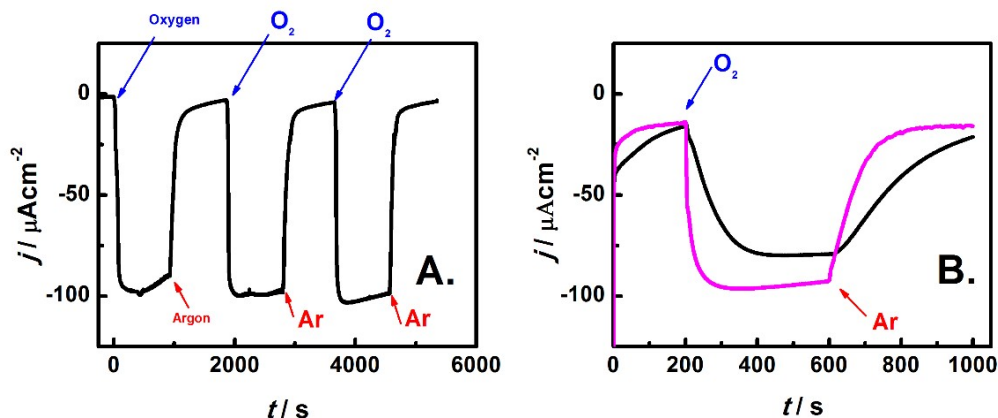
Figure 4 Chronoamperommetric responses of ITO CNP BOX electrodes for changing atmosphere. In A with 50 rpm rotation, in B comparison between with (magenta) and without rotation (black).

Comparison made from the chronoamperometric measurement (at 0.2V) shows that under rotating conditions the saturated state is attained very quickly, and for static conditions response from the electrode is much slower. Also, the response is much slower to a decrease in the oxygen level. This is because it takes time for all the oxygen to diffuse out of the matrix, and a relatively small amount of oxygen will still give a significant reduction current (The Michaelis constant of oxygen for BOx is ca 50 µM [16])..

4. **Conclusions**

In this article we have presented a simplified and flexible version of the RD method. We exemplified this by the oxygen reduction reaction catalyzed by bilirubin oxidase encapsulated together with a carbon material in a silica matrix on an ITO-coated glass electrode. Moreover, we have shown that the RD method reacts very quickly to a change in the atmosphere around the droplet. The main advantage over the regular RDE method is the possibility that any electrode material can be used as a base of working electrode for kinetic experiments and the volume which in RD method remains at least two order of magnitude smaller than in case of the standard RDE method.

5 Acknowledgements

6 References

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