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Differential Pulse Voltammetry: Evolution of an In Vivo Methodology and New Chemical Entries, A Short Review

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Background

In 1924 Heyrovsky found that current at a mercury electrode was not directly proportional to the applied voltage, but there was presence of an extra-current determined by the oxidisable chemicals present in the solution. Such extra current, that is proportional to the concentration of the compound(s) oxidized and/or reduced, is called polarographic current when obtained at a mercury electrode, is called voltammetric current when obtained at all other types of electrodes [1, 2].

Different types of voltammetric techniques are available the most common of which are chrono-amperometry linear voltammetry, cyclic voltammetry, and pulse voltammetry [3,5].

These methodologies are mainly based on the application of a “dynamic” oxidation or oxido-reduction [ox - red] potential and the resulting analysis of electrons “freed” by the chemical(s) under analysis (see Figure 1).

Technique

Voltammetric measurements are taken with a three-electrode potentiostat system made of a silver/silver chloride (Ag/AgCl) reference electrode, a copper or silver wire auxiliary (counter) electrode both approximately 100 µm in diameter and a working electrode (see Figure 2). Nowadays, the working electrode is mainly a carbon fiber micro electrode (figure 1).

Electrodes for Voltammetry

Different types of voltammetric electrodes have been developed since 1969, the most performing type appear to be the carbon based electrodes and in particular the carbon fiber - micro electrode (µCFE) (see Figure 3) [3,5,6].

The association of voltammetry with these electrodes become an electrochemical methodology

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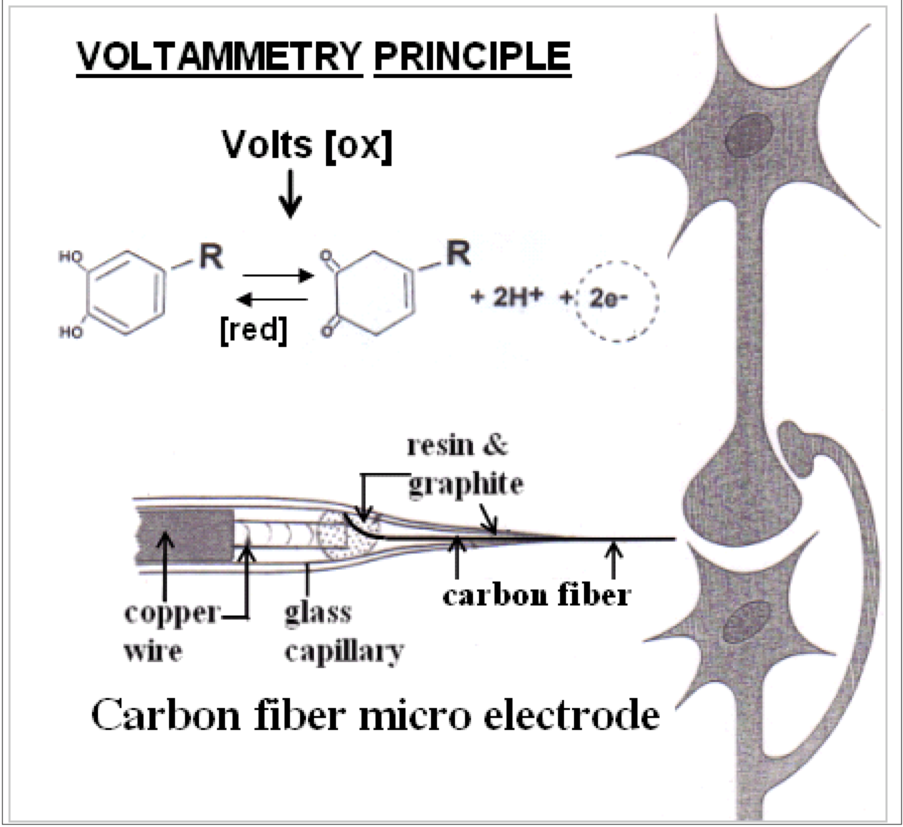

Figure 1. voltammetry principle and schematic representation of the carbon fiber micro electrode (modified from ref 5 with permission).

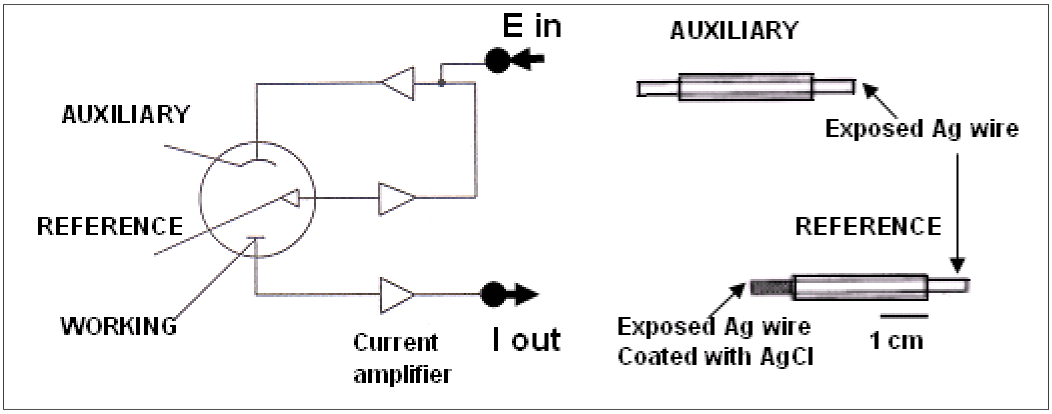

Figure 2. schematic representation of the three-electrode potential system [left] and the reference and auxiliary electrodes (modified from ref 6 with permission).

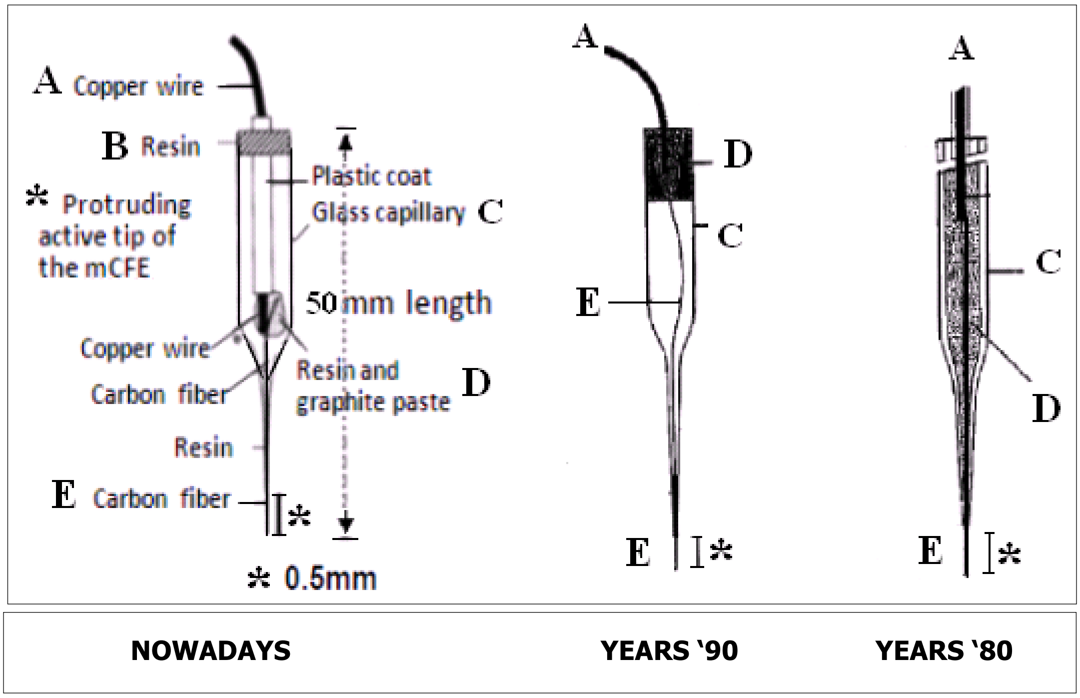

Figure 3. schematic representation of the evolution upon time of the manufacture of the carbon fiber micro electrode used as working electrode for voltammetry. A: is the conductive wire, B: resin alone or D: mixed with graphite paste. E is the carbon fiber that can vary between 6, 7, 10, 30 μm diameter. * is the protruding tip of the carbon fiber from the glass capillary (C). The length of the tip can vary from 0.1mm up to 1- 2mm in function of the size of the brain area monitored or the biological tissue analyzed, i.e. aortic(11), gastric tissue (12) (modified from ref 5 with permission).

allowing continuous, in real time and in situ detection of oxidizable chemicals.

The turning point of the use of these micro-sensors has been the application of a variety of electrical pre-treatments that are applied to the sensors before use. This has indeed improved drastically sensitivity and selectivity for analysis of electro-active chemicals and this in particular when the electrochemical methods of normal pulse as well as differential pulse voltammetry are employed [7 – 10]. Then, evolutions on pre-treatment of the μCFE have also been proposed. In particular, in addition to the electrical pre-treatment, a chemical pre-treatment consists in coating the protruding active tip of the micro sensor with Nafion (Sigma). Nafion is a sulphonated polymer repelling acids while attracting bases as it is negatively charged. This electrode is then called Nafion μCFE and it allows selective detection of dopamine and serotonin in vitro as well as in vivo with a greater sensitivity for the latter [13]. Further development of such chemical pre-treatment is the coating with a mixture of Nafion and Crown ether (Sigma). The reresulting sensor is called NaCro μCFE and shows an improved sensitivity for the selective detection of these amines [14] and in particular that of dopamine [15].

Differential Pulse Voltammetry (DPV)

DPV combines aspects of chronoamperometry and linear sweep voltammetry and exhibits high selectivity and sensitivity. Small voltage pulses of a constant amplitude (20-50 mV) are superimposed 3-5 times per second upon a linear voltage ramp (see Figure 4). The current is sampled immediately before (iA) a pulse and subtracted from the current at the end of the pulse (iB), then the difference iB - iA is expressed in terms of potential. This consents to DPV to combines the main advantage of chronoamperometry (suppression of charging current) with the resolution of

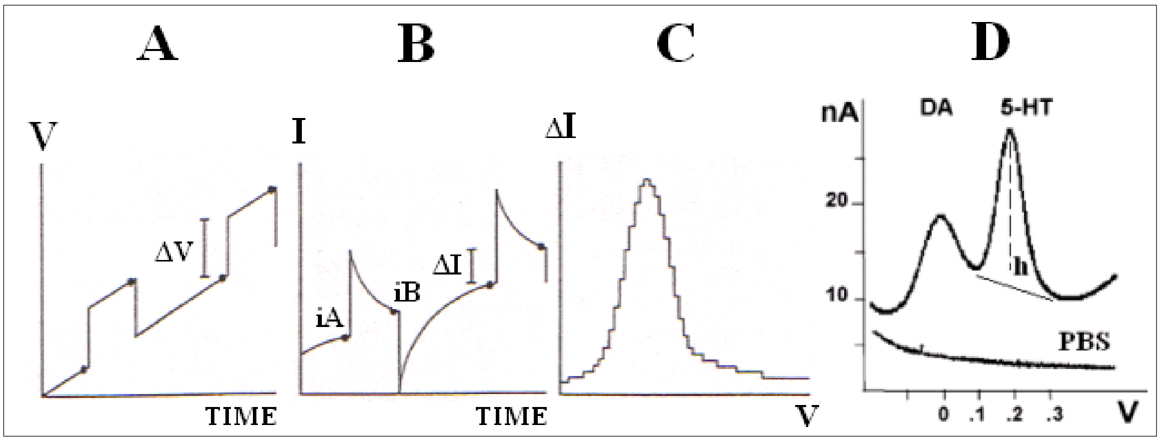

Figure 4. In Differential pulse voltammetry the applied potential is A: a linearly increasing ramp upon which small pulses of amplitude ΔV are superimposed. B: two measurements are made for each pulse; one just before the pulse [iA] and one just before the end of the single pulse [iB], to yield the differential current value ΔI. C: the differential current ΔI is reported against the applied potential V to give the peak-shaped voltammogram (peak). D: in vivo, i.e. in rat striatum, DPV monitoring the peaks of dopamine [DA] and serotonin [5-HT] at approximately 10mV and 200mV, respectively [h: size of the peak in nanoAmperes [nA].

sweep voltammetry, as it performs a local differentiation of the voltammogram obtained by linear voltammetry. The overlap between two oxidisable compounds is eliminated providing that they oxidize at sufficiently distinct potentials (at least 50-100 mV between both). Thus, the oxidation of a compound produces a sharp peak rather than the broad peak or plateau of linear sweep voltammetry, resulting in higher resolution [16].

The association of DPV with pre-treated µCFE appears to be the best methodology for rapid in situ analysis of electro-active compounds. No other combination of electrode and voltammetric method allows the same sensitivity with high resolution between oxidizable chemicals and in particular:

i) ***in vitro*****,** with the active tip of the sensor immersed in buffered solution [7, 17, 3];

ii) ***ex vivo*****,** with the active tip of the sensing electrode in contact with several tissue such as brain slices [18,19], the endothelium of rat aortic rings for detection of nitric oxide and nitrites [11, 20, 21] or in blood, and in particular in platelet-rich plasma (PRP) and/or in isolated platelet (IP) [22] as well as in gastric tissue for detection of peptides [12];

iii) ***in vivo*****,** in brain extracellular fluid when the sensor is stereotactically implanted in discrete brain areas of anesthetized as well as freely moving animals [4,10,23]. In particular, in vivo the DPV methodology associated with carbon fiber micro electrodes (DPV-µCFE) becomes an advanced approach to monitoring changes in monoamine release and their metabolism. Indeed, the method fulfills many of the criteria required to monitor specific compounds in the extracellular fluid [5] in brief:

- The undersized probe allows sampling a region of approximately 10^{-6} mm^3 volume i.e. there is high anatomical resolution of the site of measurement within discrete brain areas of rodents, with minimal damage to the nervous tissue.
- The method allows rapid, repeated measurements with accurate time resolution in vivo, in situ in real time without requiring perfusion, sample preparation, chromatographic separation or radio-labeled transmitter supplies. This is the fundamental difference between voltammetry and the perfusion techniques such as micro-dialysis [24, 25].
- The association DPV - µCFE can be performed in vivo in

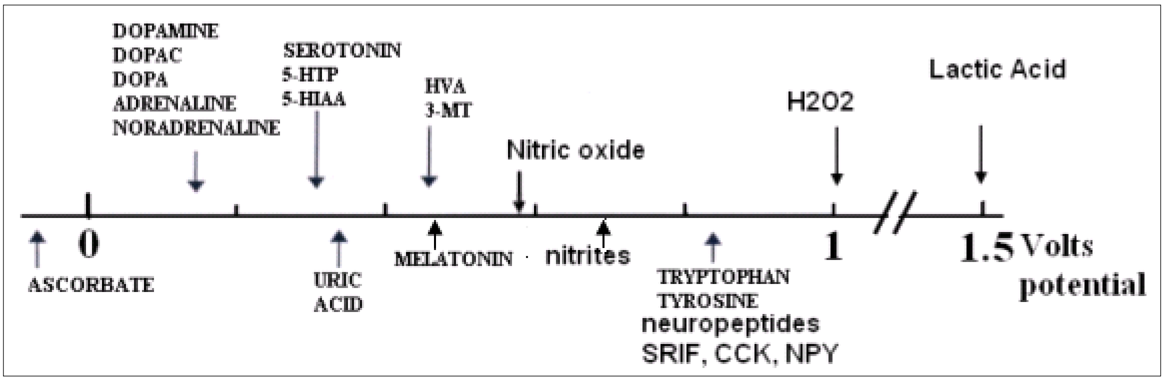

Figure 5. Electro-active compounds measurable at selective oxidation potentials in vitro as well as in vivo with the association DPV - µCFE (modified from ref 5 with permission)..

conscious freely moving animals. This solves the problems associated with anesthetics and allows correlations between neuronal activity and behavior [5, 6].

Pharmacological experiments performed with DPV - µCFE have indeed demonstrated that the following chemicals can be selectively monitored in vivo in brain areas:

- Ascorbate, noradrenaline and/or dopamine and the metabolites DOPAC, homovanillic acid, 3-methoxytyramine [26 - 29];
- Uric acid, [30];
- 5-OH-indoles (i.e.serotonin and its metabolite 5-OH-indolacetic acid) [8, 10, 13, 23].

In addition to the detection of monoamine release and their metabolism, in particular those of dopamine and serotonin, other electro-active chemicals have been successively detected with the association DPV - µCFE in vitro as well as in vivo as shown in figure 5. In particular, melatonin [31, 32] nitric oxide and nitrites [21, 33, 34] have been monitored between 500 and 700mV oxidation potential. Furthermore, neuropeptides containing electro-active amino acids such as tryptophan, cysteine, tyrosine appear to be electrochemically active in vitro; their oxidation potentials occur between +600 and +900mV [35 - 37] so that they are well demarcated from the selective DPVoltammetric oxidation peaks linked to the monoamines, the related metabolites and the other compounds mentioned above. Thus, the associated peptidergic oxidation signal has been called Peak 5 and it has been demonstrated that it is linked to the in situ oxidation of somatostatin (SRIF) [35, 37], cholecystokinin (CCK) [38 - 40] or neuropeptide Y (NPY) [41] depending on the discrete brain region analyzed. Hydrogen peroxide (H_2O_2) was also successively monitored in vivo, in situ and in real time in rat brain at approximately 1000mV [42].

Variations of the pulse polarography technique have also been proposed. In particular Differential Square Pulse Conditioning Voltammetry has been introduced since it is permitting longer “life” to the micro sensor when used in vivo [43, 44]. Another variant is Short Range Differential Pulse Polarography that couples sensitivity and selectivity with very rapid measurement of endogenous chemicals [45, 46]. Again, Differential Pulse Stripping Voltammetry, characterized by the addition to a DPV scan of a conditioning potential followed by a cleaning potential, permits nearly continuous measurements without loss of sensitivity. This is a clear advantage when one need to combine the analysis of behavior with related changes of neurotransmitters, for instance.

Finally, a very recent achievement of the association DPV - µCFE is the evidence of the feasibility of monitoring Lactic Acid both in vitro and in vivo in the frontal cortex of rodents at the selective oxidation potential +1.5 Volts [47] (see Figure 5).

It appears therefore evident that this electrochemical methodology is still evolving in detecting a variety of chemicals, at the same time as presenting a range of advantages over methods based on the preparation of samples and/or separation steps. Indeed, it allows rapid, direct, concomitant finding of different chemicals based upon specific oxidative (or red-ox) potentials either in vitro, ex vivo and in vivo conditions [48].

Such a flexibility of application is illustrated by the feasibility to couple this methodology with behavioral observations [49], with electrophysiological recordings, for example of the sleep-awake cycle [23] as well as with in vivo cell firing [36, 50, 51].

A particular example of such flexibility of utilization is the feasibility to apply the methodology in physiologic as well as pathological conditions, thus proposing selective mechanisms of actions of the neurotransmitters that can be monitored in vivo, in situ and in real time. This, taken together with the recent improvement in the methodology permitting DPVoltammetric analysis in telemetric – wireless conditions, thus allowing electrochemical studies in absolutely freely moving conditions [52] may help in the understanding of cerebral diseases and possibly in the development of pharmacological approaches to tackle them [53 – 60].

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