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Flow-injection analysis systems with different detection devices and other related techniques for the in vitro and in vivo determination of dopamine as neurotransmitter. A review

Jacobus F van Staden*, Raluca I Stefan van Staden

Process Analytical Technology Laboratory (PATLAB), National Institute of Research and Development for Electrochemistry and Condensed Matter (INCDEMC), 202 Splaiul Independentei Str., Bucharest 060021, Romania

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ABSTRACT

Dopamine (DA) is one of the most important catecholamine neurotransmitters in the human central nervous system in the brain and plays a key role in the functioning of the renal, hormonal, and cardiovascular systems. Abnormal levels of dopamine are related to neurological disorders, such as schizophrenia and Parkinson's disease and the control and fluctuations of the amount of dopamine are extremely important in monitoring with analytical systems in the human brain. This review covers the attributes of flow-injection analysis systems with different detection devices and other related techniques for the in vitro and in vivo determination of dopamine as neurotransmitter and points out the advantages and disadvantages in the implementation thereof.

1. Introductory historical background overview

1.1. Dopamine

Dopamine (DA) with IUPAC nomenclature 4-(2-aminoethyl)-benzene-1,2-diol, structure given in Fig. 1, is one of the most important catecholamine neurotransmitters in the mammalian central nervous system in the brain from where dopamine functions as a neurotransmitter that is a chemical released by the nerve cells to send signals to other nerve cells, thus acting as a chemical messenger and in such a way plays a key role in the functioning of the renal, hormonal, and cardiovascular systems. Dopamine is produced in several areas of the brain, including the substantia nigra and the ventral tegmental area, neurons containing the monoamine neurotransmitter dopamine are thus clustered in the midbrain in an area called the substantia nigra and the human brain uses five known types of dopamine receptors, labeled D_1, D_2, D_3, D_4 and D_5 [1–5].

Abnormal levels of DA are related to neurological disorders, such as schizophrenia and Parkinson's disease [1,4,5]. In Parkinson's disease, an age-related degenerative condition causing tremor and motor impairment, the dopamine-transmitting neurons in the substantia nigra area die, causing a loss of dopamine-secreting neurons in this area. As an intravenous medication dopamine acts on the sympathetic nervous system to produce effects such as increased heart rate and blood pressure. Dopamine, however, cannot cross the blood–brain barrier; therefore when dopamine is given as a drug it does not directly affect the central nervous system. Therefore, L-Dopa, that can cross the blood–brain barrier easily, and which is the precursor of dopamine is given to patients with diseases such as Parkinson's disease to increase the amount of dopamine in the brains of these patients [1–5]. Schizophrenia is often associated with elevated levels of dopamine activity in the prefrontal cortex [1].

Dopamine was first synthesized in 1910 by George Barger and James Ewens at Wellcome Laboratories in London, England [6,7]. The trivial name dopamine originates from the structure (Fig. 1), which consists of a monoamine (NH_2) side-chain linked to a catechol nucleus consisting of benzene with two hydroxyl side groups that is called *di*hydr*oxyph*enyl*a*lanine (acronym DOPA). Dopamine is formed by decarboxylation from 3,4-*di*hydroxyphenyl*a*lanine (levodopamine or L-Dopa) as a precursor (starting material) in the Barger–Ewens synthesis [6–8]. The function of dopamine as a chemical messenger as a neurotransmitter was first recognized in 1958 by Arvid Carlsson and Nils-Åke Hillarp at the Laboratory for Chemical Pharmacology of the National Heart Institute of Sweden [9,10]. For his work on dopamine, Carlsson was awarded the Nobel Prize in Physiology or Medicine in 2000 [9,11], together with co-recipients Eric Kandel and Paul Greengard.

1.2. Chromatographic methods

Gradient elution [12] and quantitative thin-layer [13] chromatography were among the first methods used in the 1960s–1970s to

* Corresponding author. Tel. +40 74 169 5743.
E-mail address: koosvanstaden@yahoo.com (J.F. van Staden).
URL: http://www.patlab.ro (J.F. van Staden).

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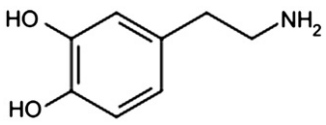

Fig. 1. Structure of dopamine.

separate and detect dopamine from other catecholamines, precursors and metabolites in an era where these techniques for liquid systems played a predominant role. In the case of gradient elution chromatography [12] the substances eluted from the column were detected and measured by either manual measurement of the optical density of the fractions at 280 μm [14] or by automatic colorimetric analysis of a part of the eluate from the column [15] or by the anodic decomposition potential of the substances [12]. A scintillation counting fluorophore was used for detection with quantitative thin-layer chromatography [13]. A gas chromatographic approach (GC) using electron capture detection (ECD) [16] was also used to separate (dopamine) and detect halogenated amine derivatives of biologically active monoamines. This was done in combination with combined gas chromatography–mass-spectrometry (GC–MS) for mass fragmentography and spectra to ensure accurate determination of different substances. The development of better instrumentation for high performance liquid chromatography (HPLC) in the 1970s enhanced the use of this analytical concept as a choice for the separation and detection of catecholamines including dopamine. The group of P.T. Kissinger in the department of chemistry at Michigan State University was especially active in this regard with a number of publications amongst them a paper describing the rapid analysis for urinary catecholamines utilizing HPLC with electrochemical detection (LCEC) [17]. The method consists of isolation and preconcentration of the catecholamines by alumina extraction followed by their rapid separation on a high performance bonded phase cation exchange resin. Norepinephrine (NE), L-dopa (DOPA), epinephrine (EPI) and dopamine (DA) were all determined in a single chromatogram in only 10 min with the considerable advantage of the determination of all four compounds in a single step. The electrochemical detection system (LCEC) has been developed by the group of Kissinger [18] as an ideal system to be used for routine clinical analysis of biological fluids since it has extreme sensitivity, good selectivity and requires only minimal sample preparation. HPLC with fluorimetric detection [19] was also described for the determination of L-dopa, noradrenalin and dopamine. The assay involved the separation of the compounds with a strong cation-exchange resin, followed by post-column derivatization with o-phthalaldehyde. The fluorescence of the derivatives was measured in a flow-through fluorimeter.

1.3. Continuous flow

The continuous flow approach originated in 1957 with the innovative pioneering paper by Skeggs [20] on automated analysis where the main instrumental development was dedicated to liquid transport in dynamic flow conditions. His design of an automatic analyzer presented several novel features on the automatic analysis of blood for urea nitrogen, glucose, calcium, chloride, alkaline phosphatase and acidity using colorimetric detection. These principles have been exploited and extended in the Technicon 'AutoAnalyzer' which dominated the continuous flow field during the 1960s and early 1970s. An automated complicated and tedious non-segmented and segmented low pressure system (the reagents were pumped by means of Technicon peristaltic pumps) was also described [21] for the resolution and analysis of catecholamines (including dopamine) and amino acids. Samples with components from tissue extracts and dissolved in acidic buffer were uploaded from an automatic sampler turntable into the manifold of the automated analyzer system and applied to the column with a pressure pump just before the column. The gradient elution program was adapted from amino acid analysis to extend the elution times in the neutral to basic region. The column flow rate was 0.6 ml/min with a back pressure of 600–800 psi and the particular system took 245 min to resolve all the components. The column effluent was first passed through a quartz flow of an UV absorptiometer prior to stream splitting to locate non-primary amino compounds. The column effluent was then splitted into 2 streams; 67% was pumped via a peristaltic pump in a non-segmented carrier stream to a scintillation counter for radioactive analysis of certain catecholamines and derivatives and 33% was pumped in an air-segmented reagent stream, passed through a short mixing coil, then debubbled and passed through a fluorometer flow cell for detection.

1.4. Flow injection

Flow-injection analysis (FIA), introduced in 1975 [22], marked an important breakthrough in unsegmented automatic continuous flow analysis and has developed over three decades as a simple, convenient, feasible analytical technique with the capability of a high sample frequency and degree of automation [23–33]. FIA is an analytical technique that is based on injecting a known volume of sample, with a well-defined shape, into a moving, unidirectional unsegmented carrier or a reagent stream. In this moving stream, the sample is physically and chemically transformed into a detectable specie that causes a detector response downstream of the injection point. If all critical parameters (reproducible injection, controlled reaction time, and controlled dispersion) are held within certain tolerance levels, the result will be reproducible [23,27]. The basic instrumentation needed for an FIA system are a multichannel pump, an injection valve, a flow-through detector, and a signal output device (originally a recorder, lately a computer). With the establishment and advances inherent in FIA, the scope and potential of the technique caught the eyes of many analytical chemists and these features make the technique very suitable over the years for satisfying the increasing demand in various fields for control and routine analysis of analytical chemistry. With their pioneering innovation Ruzicka and Hansen [22] brought an entirely new concept to analytical chemistry by creating a concentration profile (gradient) of the sample, thus, allowing any point along the gradient to be used as a potential read-out, thus exploiting transient signals. As a result of this, the kinetic nature is one of the fundamental properties of FIA. Because of the inherent dynamic nature of FIA, measurements are always performed under non-equilibrium conditions, while the system is still in its kinetic phase; it is, therefore, properly classified as a kinetic system [23,28–37]. In addition to the reaction and/or dispersion rate, kinetic aspects of FIA can be further exploited by incorporation into the system strategies which rely on controlled kinetic processes such as catalysis, stopped-flow, rate of absorption and desorption, extraction, separation, diffusion of ions or gases through membranes or packed reactors, and enzymatic reactions [22,34–38].

1.5. Sequential injection

The introduction of the sequential injection analysis (SIA) technique [30,39–42,45] broadened the scope of flow analysis. SIA is a technique that has great potential for on-line measurements due to the simplicity and convenience with which sample manipulations can be automated. The versatility of the technique is centered around a selection valve (SV) where each port of the valve allows a different operation to be performed. An important

advantage of SIA is the versatility that the multi-position valve provides [30,42–45]. Each port of the valve is dedicated to a specific purpose and the combinations of sample, standards, reagents and detectors around the valve are easily modified to suit a particular analysis. Sequential injection (SI) is now widely accepted and miniaturized in the "lab-on-valve" (LOV) format, where a microconduit monolith is mounted atop a selection valve and designed to contain all facilities for conducting chemical manipulations and even detection [33]. Microfluidic devices received a great deal of attention in recent years, becoming an emergent technology in biomedical, pharmaceutical, environmental and food analysis [46,47].

Various detection devices and other related techniques were combined with flow injection analysis and reported for the detection of dopamine.

2. Spectrophotometry

The group of Berzas Nevado [48–50] developed a number of flow-injection spectrophotometric methods for the determination of dopamine with selected catecholamines for routine analysis in pharmaceuticals. The methods of the group were based on the oxidation of catecholamine with sodium metaperiodate under various conditions. For the spectrophotometric determination of dopamine and methyl dopa with metaperiodate by flow injection analysis the authors injected samples into a sodium metaperiodate carrier solution, added an acetic acid-acetate buffer solution, pH 4.8 and pumped the solution through a 1.6 m reactor, submerged in a thermostatic water bath at 45 °C for methyldopa and 65 °C for dopamine before monitoring at 473 nm [49]. The method was applied for the determination of dopamine and methyldopa in pharmaceutical preparations. Dopamine and adrenaline solutions are hydrolyzed in alkaline medium and oxidized by atmospheric oxygen to give yellow-brownish colorations due to the amino derivatives and as these compounds are very unstable, the hydrodynamic nature of FIA was used by the group of Berzas Nevado [50] to avoid the instability problem and to achieve reproducible spectrophotometric measurements (λ=390 nm) of the aminochrome in alkaline medium. Calibration graphs were linear up to 2×10^{-4} mol/l with quantification limits of 2.5×10^{-6} mol/l and 3.3×10^{-6} mol/l for dopamine and adrenaline respectively. The method was applied for the determination of dopamine and adrenaline in pharmaceutical preparations. It is clear from the results given in these publications that the spectrophotometric FIA methods will be difficult to apply for the determination of dopamine in biomedical applications where the whole range of neurotransmitter catecholamines is present.

3. Ion selective electrodes (ISE)

Suitable open-tubular potentiometric ion-selective electrodes coupled with flow injection analysis (FIA) may be very attractive for the selective determination of dopamine as a neurotransmitter in biomedical applications due to its desirable characteristics, such as low consumption of reagents, high sample throughput, real-time analysis, simple automated operation, and possibility of on-line sample pretreatment [51,52], if the contribution of memory effect or response time phenomena especially on the tailing edges of ISEs can be controlled and minimized [53].

Montenegro and Sales [54] developed an FIA method for the indirect determination of dopamine in injections based on its oxidation with periodates using a periodate-selective electrode. An electrode with a tubular configuration, no internal reference solution, and a PVC (31.2%) membrane, with metaperiodate bis(triphenylphosphoranylidene) ammonium (1.3%) as ion exchanger and 2-nitrophenyloctylether (67.5%) as a mediator solvent, was used. Optimization procedures were directed at potentials versus dopamine readings instead of potential versus the remaining IO_4^-. This approach was achieved by selecting a 50 cm reactor and an overall flow of 7 ml/min, and injecting 70 μl of dopamine standards into a 3.0×10^{-4} mol/l IO_4^- solution. Under these conditions, a linearity range of 8.0×10^{-3} to 2.7×10^{-1} g/l, with a slope of 310.1 ± 7.4 mV l/g and a reproducibility of ±0.4 mV, were recorded (n=8). Interference from common excipients was negligible. Under these conditions, analysis of dopamine injections (n=12) presenting 200 mg/injection gave average and standard deviation values of 201.0 and 3.3 mg/injection, respectively. The simple and inexpensive flow-injection analysis (FIA) manifold, with a good potentiometric detector, enabled the analysis of 200 samples/h without requiring pretreatment procedures. Wolyniec et al. [55] described batch and flow injection systems for the determination of catecholamines based on ion-pairs of epinephrine (EP), dopamine (DA), and L-Dopa (LD) as ion selective electrodes with tetraphenylborate (TPhB) with the incorporation of EP-TPhB, DA-TPhB, or LD-TPhB ion exchangers in poly(vinylchloride) (PVC) membranes plasticized with di(2-ethylhexyl)sebacate (DES). The electrodes show a near-Nernstian response in the concentration ranges: 1.0×10^{-4}–1.0×10^{-2} mol/l (epinephrine), 5.0×10^{-5}–1.0×10^{-2} mol/l (dopamine), and 5.0×10^{-4}–1.0×10^{-2} mol/l (L-Dopa) and were applied in pharmaceutical preparations. A polymeric membrane permanganate-selective electrode has been developed as a current-controlled reagent release system for potentiometric detection of ascorbate, dopamine and norepinephrine as reductants in flow injection analysis [56]. An external current was applied to control the diffusion of permanganate ions across the polymeric membrane precisely. The permanganate ions released at the sample–membrane interface from the inner filling solution of the electrode were consumed by reaction with the analyte reductant (ascorbate, dopamine or norepinephrine) in the sample solution with the result that the measured membrane potential was changed sensing the selected analyte reductant potentiometrically. The potential peak heights were proportional to the reductant concentrations in the ranges of 1.0×10^{-5}–2.5×10^{-7} mol/l for ascorbate, 1.0×10^{-5}–5.0×10^{-7} mol/l for dopamine, and 1.0×10^{-5}–5.0×10^{-7} mol/l for norepinephrine, respectively with the corresponding detection limits (3σ) of 7.8×10^{-8}, 1.0×10^{-7} and 1.0×10^{-7} mol/l. The potential peak heights showed relative standard deviations of 3.1%, 4.2% and 3.8% (n=6) for ascorbate, dopamine and norepinephrine respectively. The proposed system was applied for the determination of ascorbate, dopamine and norepinephrine in pharmaceutical preparations and vegetables with a recovery of better than 95%.

4. Amperometry

Amperometric measurements under the hydrodynamic conditions in flow injection analysis or even liquid chromatography should be particularly beneficial and attractive for analytical laboratories and especially in biomedical applications since the fouling problems are not severe as in batch experiments. Furthermore this is especially useful and attractive for routine analytical laboratories where the concentrations of the analytes are very low. Flow injection analysis with a poly(3-methylthiophene), PMeT, as the electronically conducting polymer modified electrode serving as an amperometric detector, were performed for the determination of catechol, ascorbic acid, dopamine, epinephrine, NADH, p-aminophenol and acetaminophen using Sörensen buffer as the mobile phase [57]. The modified electrode displayed

excellent response stability for successive injections and detection limits were 10 μg/l for catechol, dopamine, epinephrine, NADH and p-aminophenol, 1 μg/l for acetaminophen and 100 μg/l for ascorbic acid. Voltammetric peak positions were affected by the nature of the electrolyte and its pH. McKean and Curran [58] implemented cross-correlation for flow injection analysis by using two parallel flow lines, each with amperometric detectors, and driven by peristaltic pumps (Fig. 2). One flow line was used to generate the reference signal for an analog correlator circuit and the other to generate the analyte signal. Cross-correlation was performed by multiplying these signals together at a time delay of zero, followed by low pass filtering. The outputs from the potentiostat and the bipotentiostat were cross-correlated with analog circuitry and the correlation signal measured on a strip chart recorder. Using dopamine as the test system, improvements in signal-to-noise ratios of about two orders of magnitude were found for the correlation signal over the direct measurement of the electrode current. A flow injection analysis system for the determination of dopamine was also used together with an amperometric biosensor developed with the incorporation of an enzyme into a solid-paraffin–graphite-particle-matrix [59]. Tyrosinase served as a model enzyme and the biosensor response was characterized with respect to its response to dopamine. The electrode response was fast, reversible and linear in a large domain of 0.1 μ mol/l–1 m mol/l. The enzyme-solid paraffin carbon paste electrode showed markedly improved stability in flow injection analysis compared to the classical liquid-paraffin–graphite-based biosensors. A sampling rate of 79 samples per hour was obtained with the FIA-biosensor system with an RSD of 2.2% ($N=63$) with a detection limit of 50 nmol/l for dopamine.

The entry of molecules into brain tissue is minimized by the blood brain barrier (BBB). However the ability to study the transport of molecules across the BBB and the resultant release of neurotransmitters and/or neuropeptides is challenging and a number of techniques [60] have been developed to help describe and characterize the complexity of brain chemistry. Unfortunately, an underlying theme in many of the techniques is the isolation of the two main components of the BBB, namely, blood flowing through a vessel containing endothelial cells and the neuronal network responsible for secretion of the neurotransmitters and neuropeptides into the extracellular environment. In an attempt to combine pharmacological studies with physiology, the group of Dana Spence [61,62] developed a flow-based system that serves as a novel tool for investigations involving the microcirculation. With this work the group of Dana Spence marked an important breakthrough and brought an entirely new concept to analytical chemistry with the combination of pharmacological studies with physiology in flow-systems. They specifically developed a system that allows for the quantitative monitoring of flow-induced ATP release from rabbit erythrocytes as they traverse microbore tubing whose diameter approximates that of resistance vessels in vivo. They also found that the ATP release is purely a physiological event since no agonists were added to the erythrocytes to evoke the ATP release. In addition, the erythrocytes were not lysed during the flow process. In order to improve this microcirculation model, endothelial cells were cultured and immobilized onto the lumen of microbore tubing coated with fibronectin [63]. In the presence of ATP, these coated endothelial cells produced and released nitric oxide (NO), which was detected amperometrically in a parallel flow cell. In an effort to improve blood brain barrier (BBB) models, the group of Dana Spence [60] described the culturing of rat pheochromocytoma (PC 12) cells, their subsequent immobilization to the lumen of microbore tubing coated with poly-L-lysine and the flow-based amperometric detection of dopamine in an immobilized cell reactor as outlined and described in an experimental setup as reproduced in Fig. 3 from Ref. [60].The system shown in Fig. 3 was used to determine Ca^{2+}-induced PC 12-derived dopamine where the microbore tubing labeled as "C" was coated with PC 12 cells. The authors described [60] a protocol for immobilizing PC 12 cells onto the lumen of fused silica microbore tubing having an inside diameter of 250 μm and coated with a thin layer of poly-L-lysine from a 50 μg/ml solution of poly-L-lysine. Attachment of the cells to poly-L-lysine took approximately 2 h and could be maintained inside the tubing for a period of 5 d. When placed in a continuous flow system, the immobilized cells released neurotransmitters when stimulated by Ca^{2+}-ions. Results of repetitive injections of 500 nl of a 6 mmol/l dopamine into the continuous flow system gave an RSD of 2.3%. Injection of dopamine standards ranging in concentrations from 1 to 25 mmol/l as control into a (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES) buffer gave results from linear regression analysis with a slope of 1.02×10^{-9} A/mol/l and a y-intercept of 2.13×10^{-9} ($r^2=0.9869$) A.

Metrohm glassy carbon electrodes as working and auxiliary electrode, respectively with an Ag–AgCl Metrohm reference electrode formed the basic detection system in the flow injection amperometric determination of L-dopa, epinephrine or dopamine in pharmaceutical preparations [64]. The FIA system enabled a sampling rate of 100 samples/h in the concentration ranges of

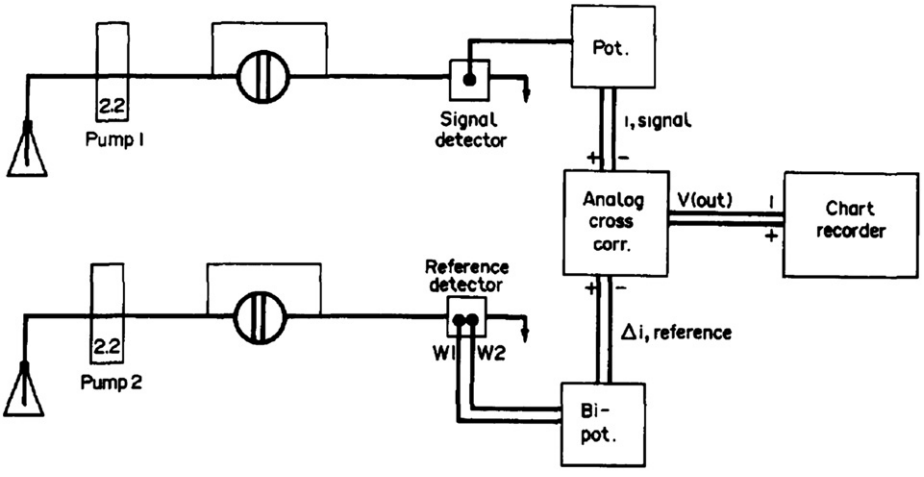

Fig. 2. Schematic diagram of the parallel stream flow injection analysis system with cross correlation in real time. Reproduced from Ref. [58].

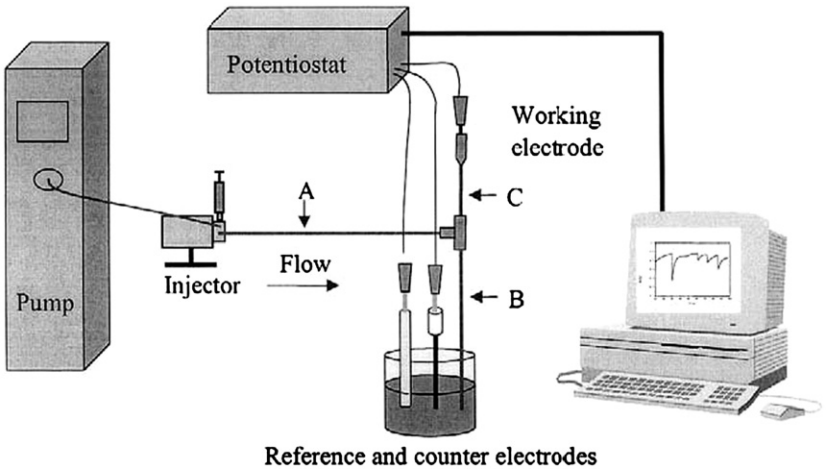

Fig. 3. Experimental setup for measuring dopamine standards and PC 12-derived dopamine. A syringe pump delivered HBSS to a 4-port injection valve fitted with a 500 nl internal rotor. The flow was sent through the tubing section labeled 'A' and then split into two separate streams. One stream was sent to a reservoir containing a reference and counter electrode in HBSS (B), while the other stream (C), traveling through a section of tubing with immobilized cells, was sent to a Pt working electrode. The surface of the electrode was placed in the terminus of tubing section 'C' in order to measure Ca^{2+}-induced dopamine release from the immobilized PC 12 cells. The flow was split into separate streams for easy manipulation of the reference, counter, and working electrodes. Reproduced from Ref. [60].

7×10^{-6}–5×10^{-5} mol/l with a straight line for L-dopa (r=0.998; n=5), epinephrine (r=0.998; n=5) and dopamine (r=0.997; n=5) with an RSD for L-dopa, dopamine and epinephrine of 1.9%, 0.7% and 1.4% respectively and a recovery of between 98% and 102% for commercial pharmaceutical preparations. An FIA system with amperometric detection for the determination of dopamine in pharmaceutical products was established using a polyphenol oxidase biosensor obtained from soursop pulp [65]. The flow amperometric detection was carried out at a potential of 0.10 V (vs. Ag/AgCl) with the injection of 250 ml sample into a 0.3 mol/l phosphate buffer carrier solution at pH 7.8 with a flow rate of 2.5 ml/min. The developed biosensor showed good stability and reproducibility at a sampling rate of 40 samples/h and was stable for up to 500 determinations in 60 d, without considerable loss of enzymatic activity. The FIA system gave a linear response for dopamine in the concentration range from 2×10^{-2} to 2×10^{-4} mol/l, with RSD better than 3.4% for the determination of dopamine in different commercially pharmaceutical formulations. Voltammetry and flow-injection amperometry was also used in the indirect determination of dopamine with an electroanalytical approach based on an electro-generated addition product with the determination of dopamine in the presence of sodium 4-aminobenzenesulfonate as a nucleophile [66]. Dopamine was pre-oxidized, followed by addition attack of electro-inactive sodium 4-aminobenzenesulfonate (ABSA) forming an addition product in the oxidized state. The oxidized adduct was used to monitor dopamine. The flow-injection amperometric system showed a linear response between 0.4 and 20 μmol/l for dopamine with a slope of 14.2 nA/μmol/l at a flow rate of 300 μl/min of a 4.0 mmol/l ABSA as a carrier solution and a pH 4 buffer solution. The detection limit (S/N =3) was 0.05 mmol/l and the RSD was 0.2% for n=10 of 4 mmol/l standard dopamine solutions. The working system was highly stable for a continuous flow for 5 h without considerable loss of the reduction current. The authors claimed that the performance is comparable to the reported flow amperometry using a carbon nanotube-modified electrode [67] and is better than using a biosensor [65].

5. Fast-scan cyclic voltammetry

Mark Wightman [68,69] started his research on the development of in vivo voltammetry in the early to mid 1970s with the direct and continuous measurement of the release of neurotransmitter metabolites in cerebrospinal fluid (CSF) after electrical stimulation or pharmacological manipulations of neural pathways. This measurement was very simple in principle. A potential was applied to a micro carbon electrode stereotaxically placed in a lateral ventricle. One then measured the minute current which resulted from the oxidation or reduction of small molecular weight constituents near the sensor electrode. The potential at which the electrolysis occurred served as a qualitative indication of the constituent being monitored and the current itself was directly proportional to the concentration of this component in CSF. The results of the in vivo voltammetric monitoring of dopamine metabolites in CSF following release by electrical stimulation [69] were verified by independent liquid chromatography. Selectivity plays a very important role in the accurate electrochemical determination of specific analytes in a complex matrix especially in the in vitro electrochemical assay of specific neurotransmitters in the brain. A study of the in vitro comparison of the selectivity of different types of electrodes for in vivo electrochemistry was the next logic step in this regard [70]. This work by the group of Wightman also formed part of the development of ultramicroelectrodes for use as in vivo probes [70,71], that are so essential in this regard and this work marked an important milestone for the future. Microvoltammetric electrodes were used to monitor dopamine released in the caudate nucleus of the rat after electrical stimulation of the medial forebrain bundle [71] and also diffusion processes in brain tissue [72]. From my viewpoint the development of a small volume electrochemical detector for micro-column liquid chromatography [73] and the determination of dopamine in the brain with micro-voltammetric electrodes in 1980 [74,75] was one of the first breakthroughs in the real miniaturization of detection devices. Their results were further improved by polishing the surface of the ultramicrovoltammetric electrodes to a bevelled tip, improving the electrode properties, while at the same time made the preparation of the electrodes easier with the result of forming an electrode practical for routine use [76]. Fast scan cyclic voltammetry [77] with a homebuilt potentiostat and microvoltammetric electrodes with radii smaller than 7 μm [78] was used by the group to study the scan rate dependence of these electrodes in order to improve the quality of voltammetric data by repairing electrode/insulator defects of these electrodes. Flow injection analysis was introduced in the mid 1980s into their system [79] when micro-voltammetric electrodes similar to those previously described [74] were used to study the dispersion processes that occur with loop injectors and channel-type electrochemical detectors using dopamine as a test compound. They found

that the primary source of dispersion came from the transport tubing with the finite volume of the channel-type detector contributed negligibly to the observe response. In a study of temporal characterization of perfluorinated ion-exchange coated micro-voltammetric electrodes for in vivo use, the temporal response of a Nafion-coated carbon-fiber voltammetric micro-electrode to the rapid application of electroactive species was measured by flow injection analysis or by fast-scanning cyclic voltammetry [80]. Very thin Nafion-films smaller than 200 nm were applied to allow rapid permeation of dopamine so that distortion of the concentration pulse was not apparent. The results revealed that the electrodes responded rapidly to the stimulated secretion of dopamine in the brain of a rat so that they were suitable as in vivo probes of neurotransmitter release. The films furthermore protected the electrode surface and thus prevented diminution of the voltammetric signal. In a further development fast-scan voltammetry was employed when cyclic voltammograms were recorded in a flow-injection system at a scan rate of 200 V/s at carbon-fiber electrodes with and without a coating of perfluorinated ion exchanger [81]. Voltammograms for uncoated electrodes gave peak currents for dopamine that were larger than expected with the result that sub-micromolar amounts of dopamine could be detected. Fast-scan cyclic voltammetry was extended to the simultaneous electrochemical determination of dopamine and dissolved molecular oxygen (O_2) in vivo in flow-injection analysis with the injection of a solution containing dopamine and oxygen into a carrier stream of deaerated buffer containing 150.0 mmol/l NaCl and 20 mmol/l HEPES at pH 7.4 with a flow rate of 1.91 ml/min [82]. Glass and stainless steel components were used instead of Teflon tubing and components because the latter are permeable to O_2. In flow injection analysis the carbon-fiber disk electrodes coated with a perfluorinated ion-exchange material responded to step changes in dopamine and O_2 with a half-rise time of less than 200 ms as shown in Fig. 4. The results revealed that the electrodes maintain a stable response in vivo for at least 6 h and the carbon-fiber disk electrodes coated with Nafion have shown to be a suitable sensor to measure rapid and independent changes in dopamine and O_2 levels in the extracellular fluid of the caudate nucleas of an anesthetized rat in response to an electrical stimulus. The use of fast-scan cyclic voltammetry gave a method with high spatial resolution and time resolution of the order of 100 ms.

Constant potential amperometry with Nafion-coated carbon-fiber electrodes were compared with fast-scan cyclic voltammetry for the same electrode with a flow injection system for the in vivo monitoring of the neurotransmitter dopamine dynamics in the rat brain during release of dopamine into the extracellular space of the brain during electrical stimulation of neurons [83]. The data revealed that constant potential amperometry is a viable technique for the detection of low concentrations of dopamine. Dopamine permeates the film more quickly with constant-potential amperometry than with repeated fast-scan cyclic voltammetry as predicted by diffusion equations resulted in a more rapid amperometric response, but interpretation of the in vivo data based on in vitro calibration was more difficult. It was further demonstrated that for cyclic voltammetry the temporal delay caused by diffusion through Nafion film can be removed by deconvolution procedures. Although constant potential amperometry was more suitable as an in vivo monitoring technique, comparison had shown that it had the disadvantage when compared to fast-scan cyclic voltammetry, because the diffusion layer was less well defined. The next step of the group was to improve the data acquisition of their fast-scan cyclic voltammetric flow injection system [84] to reduce noise due to the requirements of high sensitivity and rapid measurements demand and because of the small faradaic currents generated when monitoring biological samples for dopamine with fast-scan-cyclic voltammetry and to allow observation of low concentrations of neurotransmitters.

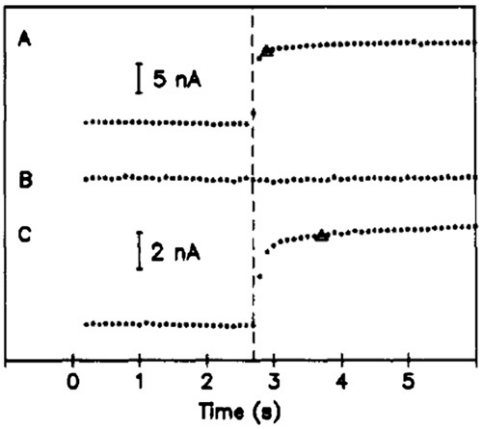

Fig. 4. Response of a Nafion-coated carbon-fiber electrode to a simultaneous step change in dopamine and O_2. The cyclic voltammograms were collected at 100-ms intervals and each point represents the result from a single voltammogram. The O_2 profile (A) was obtained by plotting the current measured in a window from −1.20 to −1.40 V on the cathodic scan. The dopamine plot (C) was constructed similarly with data from 0.55 to 0.75 V on the initial anodic sweep of each cyclic voltammogram. The middle trace (B) is plotted as a control based on data from 0.00 to 0.20 V on the initial anodic scan. The dashed line is the time at which O_2 first deviates from the baseline. Triangle=90% response. Reproduced from Ref. [82].

They succeeded to write software programs in LabVIEW and Labmaster with a single acquisition board to generate the voltage waveform and collect the current eliminated irregularities caused by independent timing and a phase-locked loop eliminated noise attributed to frequency drift. A 300 V/s triangle waveform from a rest potential of −400 to 1000 mV and back to −400 mV was normally used for fast-scan cyclic voltammetry with a repetition rate of 10 Hz, voltages being relative to an Ag/AgCl reference electrode. For fast-scan cyclic voltammetry of mast cells, voltage was scanned from 0.1 to 1.4 to 0.1 V at 800 V/s, the waveform repeated every 33 ms. A system for the subsecond adsorption and desorption of dopamine at carbon-fiber microelectrodes was developed [85]. High-repetition fast scan cyclic voltammetry and chronoamperometry were used to quantify and characterize the kinetics of dopamine and dopamine-o-quinone adsorption and desorption at carbon-fiber microelectrodes. A flow injection analysis system was used for the precise introduction and removal of a bolus of electroactive substance on a sub-second time scale to the disk-shaped surface of a microelectrode that was fabricated from a single carbon fiber. The new methodologies allowed for more accurate determinations of the kinetics of neurotransmitter release events (10–500 ms) systems.

It has been shown by Howell and Wightman [78] that fast-scan cyclic voltammetry is useful to follow chemical changes, it has been used in a variety of applications and has been shown to be particularly useful for monitoring fluctuations of neurotransmitter concentration such as dopamine in vitro [86,87] and in vivo [88–90]. Micro-voltammetric electrodes were used in these applications, because small currents are generated even at high scan rates, which minimized distortion of the cyclic voltammograms by ohmic drop. A limitation of fast-scan cyclic voltammetry is that the background currently greatly exceeded the faradaic current from redox reactions of species in solution giving large amplitude of the background current relative to the currents for the solution species of interest [92]. The background is composed of current required to charge the double layer and current arising from redox reactions of surface-attached functional groups. The magnitude of both these sources is directly proportional to scan rate, whereas the current arising from a diffusion-controlled electrochemical reaction is proportional to

the square root of the scan rate [91]. The background furthermore tends to drift and this drift limits the use of digital background subtraction techniques to less than 90 s before distortion of dopamine signals occurs. Therefore, optimum ratios of the faradaic to background current are not achieved with fast-scan cyclic voltammetry with micro-voltammetric electrodes [92]. The group of Wightman [92] presented a particularly useful improved background subtraction approach to minimize the impact of the background, termed analog background subtraction. They recorded the background, and played its inverse back to the current transducer during data acquisition so that it canceled the background in subsequent scans. Although background drift still occurred and was recorded, the drift magnitude was small compared to the original background. The advantages of their improved method were that it allowed the use of higher gains in the current transducer, minimizing quantization noise and as the background amplitude was greatly reduced, principal component regression (PCR) could be used to separate the contributions from drift, dopamine and pH when appropriate calibrations were performed. They demonstrated the use of their approach with several applications, for example by monitoring transient dopamine fluctuations for 15 min in a flow injection system.

Although the fast-scan cyclic voltammetric technique has advantages over many electrochemical techniques as it provides an analyte-selective response with subsecond temporal resolution [90], the technique had not been linked with microfabricated microelectrode arrays (MEAs) [93]. In a very successful pioneered innovated breakthrough presentation [93], microfabricated microelectrode arrays were made from pyrolyzed photoresist films and compared to PAN-type (T-650) carbon films using fast-scan cyclic voltammetry to characterize simultaneous measurements of important biological analytes. The pyrolyzed carbon MEAs were then used in various applications with flow injection systems. In their first application they showed (Fig. 5) the simultaneous detection of dopamine at four spatially different locations, 100 μm apart. Measurements were done at 400 V/s and 10 Hz in Tris buffer, pH 7.4. Upon injection of the dopamine solution into the pyrolyzed photoresist films (PPF) arrays, nearly identical electrochemical responses were recorded at each electrode in the array as given in Fig. 5. In their second application they demonstrated the concurrent use of amperometric and fast-scan cyclic voltammetry with combined advantages of both techniques with the simultaneous detection of both techniques. The fast-scan cyclic voltammetry was performed at 400 V/s at 10 Hz. By collecting the amperometric data (E_{app}=0.8 V) as well as the cyclic voltammograms simultaneously, they were able to see both diffusion and adsorption-controlled processes in real time. Both normalized signals were in response to a 5 s, 1 μmol/l injection of dopamine in Tris buffer, pH, 7.4. The rise and fall time response, τ_{90}, was measured to be 0.8 and 0.7 s for amperometry and 1.9 and 2.1 s for fast-scan cyclic voltammetry. The sensitive values were 12 ρA/μmol/l for amperometry and 5 nA/μmol/l for fast-scan cyclic voltammetry. In their third application they successfully accomplished the simultaneous detection of different analytes, oxygen and dopamine, with decoupling of electrode signals as shown in Fig. 6. They reported a 30-fold increase in sensitivity to dopamine (9.0×10^{-3} (nA/μ mol/l μm^2)) as well as a 3-fold increase in the sensitivity for oxygen (2.1×10^{-4} (nA/μ mol/l μm^2)) compared to the work by Zimmerman et al. [82].

6. Microchip-based microfluidic systems

Wang et al. [94] developed a microchip-based flow injection amperometric system for the automatic, rapid and selective determination of dopamine in the presence of ascorbic acid. The system presented, was composed of a polycarbonate microfluidic chip with an electrochemical detector, a gravity pump, and an automatic sample loading and injection unit. The selectivity of the electrochemical detector was improved by modification of the gold working microelectrode, which was fabricated on the polycarbonate chip by UV-directed electroless gold plating with a self-assembled monolayer (SAM) of 3-mercaptopropionic acid (MPA). Postplating treatment methods for cleaning the surface of electroless gold microelectrodes were investigated to ensure the formation of high quality SAMs. The influence of detection

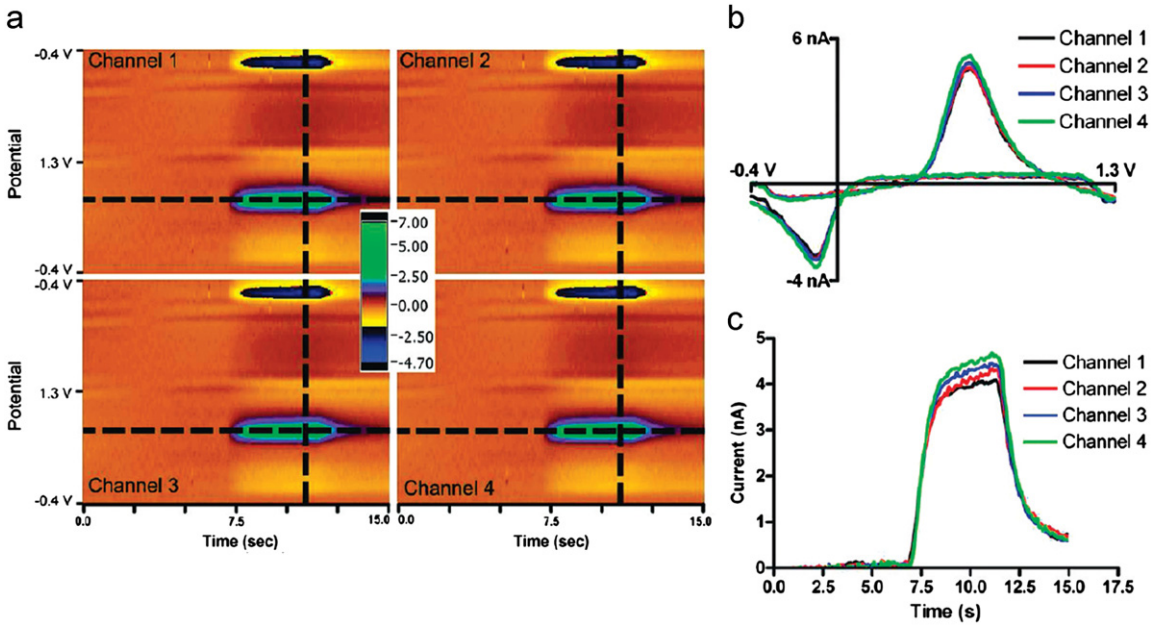

Fig. 5. Simultaneous dopamine detection. (a) Color plots collected concurrently on adjacent electrodes. (b) Simultaneous cyclic voltammograms of 1 μ mol/l dopamine (from the vertical dashed line). (c) Simultaneous peak voltammetric current versus time traces for a 5 s injection of 1 μ mol/l dopamine (from the horizontal line). Measurements were done at 400 V/s and 10 Hz in Tris buffer, pH 7.4. Reproduced from Ref. [93]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

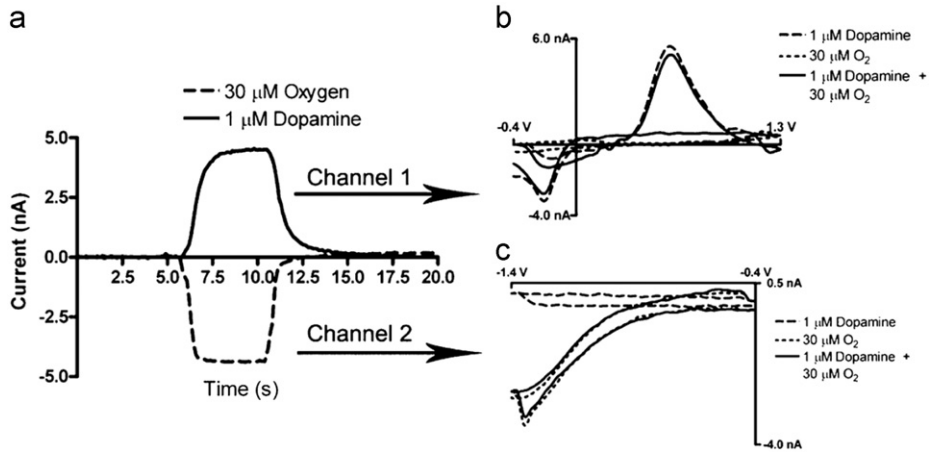

Fig. 6. Simultaneous detection of dopamine and oxygen. (a) Average of five peak current vs. time traces depicting the simultaneous detection of physiologically relevant concentrations of dopamine and oxygen (5 s injection of both 1 μmol/l dopamine and 30 μmol/l oxygen). (b, c) Simultaneous cyclic voltammograms recorded using dopamine and oxygen waveforms: (dashed line) 1 μmol/l dopamine, (dotted line) 30 μmol/l oxygen, (solid line) 1 μmol/l dopamine and 30 μmol/l oxygen. Data collected at 400 V/s for dopamine and 200 V/s for oxygen at 10 Hz. Experiment performed in Tris-buffer (pH 7.4). Reproduced from Ref. [93].

potential, flow rate, and sampling volume on the performance of the chip-based FIA system was studied. The analytical performance of the microchip-based flow-injection amperometric system is illustrated by typical recorder traces of flow injection peak signals for series of dopamine standard solutions in Fig. 7. The authors claimed recoveries of better than 95% for spiked urine samples with dopamine concentrations ranging from 10 to 200 μmol/l, achieved a detection limit of 74 nmol/l for dopamine at a sample throughput rate of 180 per hour and an RSD of 0.9% for peak heights for 19 runs of a 100 μmol/l dopamine solution. Li and Martin [95] described the fabrication and evaluation of a multilayer microchip for quantitatively measuring the amount of catecholamines released from PC 12 cells immobilized within the same device.

Through the action of poly(dimethylsiloxane) (PDMS)-based pneumatic valves [96], samples from the hydrodynamic region were injected into the electrophoresis portion of the microchip, where the analytes were separated based upon mass and charge. Post-separation derivatization using naphthalene-2,3-dicarboxaldehyde (NDA) and 2-β-mercaptoethanol (2ME) enabled fluorescent detection of the catecholamines. Following the optimization of the separation/detection portion of the device, off-chip cell stimulation experiments were performed to demonstrate the ability of the device to detect dopamine from a population of PC 12 cells. The final 3-dimensional device that integrates an immobilized PC 12 cell reactor with the bilayer continuous flow sampling/electrophoresis microchip was used to continuously monitor the on-chip stimulated release of dopamine from PC 12 cells.

7. Chemiluminescence and electrochemiluminescence

Chemiluminescence is the emission of energy with limited emission of heat (light, luminescence), as the result of a chemical reaction, the involvement of luminol is well known and it is nowadays a common analytical technique for various detection assays in biochemical applications. Chemiluminescence is a high sensitive detection technique and should be easily combined with flow injection analysis to provide rapid and sensitive signals in the determination of catecholamines, but the use of flow injection analysis with chemiluminescence as detection is rather rare [97,98]. Deftereos et al. [97] utilized chemiluminogenic oxidation of catecholamines with potassium permanganate in acidic medium and reported the determination of 0.05–1.0 μg/ml of epinephrine and L-dopa and 0.1–1.0 μg/ml of norepinephrine and dopamine with a sampling rate of 80 samples per hour. Lucigenin chemiluminescence was used by Al-Warthan et al. [98] for the FIA determination of isoprenaline and the logarithmic calibration curve was linear over the range 10^{-7}–10^{-4} mol/l Zhang et al. [99] described an FIA system for the determination of trace amounts of dopamine based on the inhibition effect of dopamine on the iron(II)-induced chemiluminescence (CL) of 10,10′-dimethyl-9,9′-biacridinium dinitrate (lucigenin). The presence of a non-ionic surfactant, polyoxyethylene (23) lauryl ether (Brij 35) caused an increased in the inhibition effect. The proposed FIA/CL system was suitable to determine dopamine over the range 1×10^{-8}–2×10^{-7} mol/l with an RSD of 0.7% for eight determinations of 6×10^{-8} mol/l dopamine and a detection limit ($S/N=3$) of 2×10^{-9} mol/l at a sampling rate of 40 samples per hour. Li et al. [100] presented a plant tissue-based chemiluminescence flow biosensor/FIA system for the determination of unbound dopamine in rabbit blood with on-line microdialysis sampling where potato roots acted as molecular recognition element in the detection system. Dopamine was oxidized by oxygen under the catalysis of polyphenol oxidase in the tissue column to produce hydrogen peroxide that reacted with luminol in the presence of peroxidase to generate a CL signal. The CL emission intensity was linear with dopamine concentration in the range 1×10^{-5}–1×10^{-7} g/ml with a detection limit of 5.3×10^{-8} g/ml (3σ) and an RSD of 1.7%. Combined with microdialysis sampling, the biosensor was applied to in vivo on-line monitoring of the variation of dopamine level in rabbit blood. Flow injection was also coupled with luminol-hexacyanoferrate(III) chemiluminescence detection where the determination of dopamine was based on the inhibition of the intensity of CL from the luminol-hexacyanoferrate(III) system in basic medium [101]. The method was applied for the determination of dopamine in commercial pharmaceutical injection samples and was suitable for the determination of dopamine over the range 30–100 μg/l and 400–3000 μg/l, with an RSD of 2.32% for 70 μg/l dopamine and 1.22% for 1500 μg/l dopamine ($n=20$) and a detection limit of 5 μg/l at a sampling rate of 135 samples/h. An FIA/CL system with similar chemiluminescence detection was also presented [102]. The determination of catecholamines (dopamine) by FIA/CL based on the inhibition on the chemiluminescence reaction of luminol-potassium chlorate [103] and luminol-potassium periodate [104,105] in basic solutions was also described.

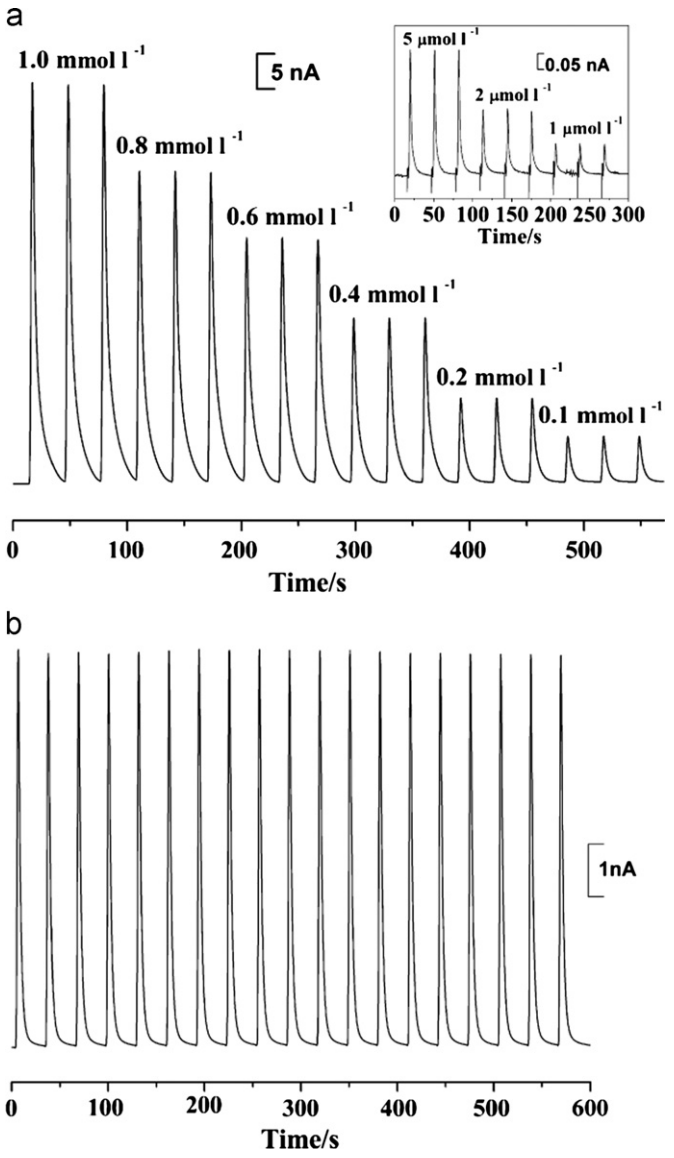

Fig. 7. (a) Typical recording traces for a series of dopamine standard solutions in the concentration range of 0.1–1 mmol/l. Inset: recording traces for dopamine standard solutions of 1 µmol/l, 2 µ mol/l and 5 µmol/l. (b) A typical recording trace for 19 consecutive runs of a 100 µmol/l dopamine standard solution. Experimental conditions: detection potential: 0.2 V vs. Ag/AgCl. Reproduced from Ref. [94]

Electrochemiluminescence (ECL) is a form of CL in which the light emission is generated by electrolysis [106]. ECL not only retains the advantages of conventional methods, but also offers several additional merits such as temporal and spatial control over the CL reaction [107]. The ECL reaction can be controlled at will by alterations to the applied potential on the electrode because at least one of the reagents participating in the ECL reaction is produced in situ at the electrode surface. The light emission is concentrated close to the electrode surface, which can be shaped and accurately positioned in relation to the optical measurement for maximum sensitivity. An FIA/ECL system was described for the determination of dopamine based on its inhibition of the electrochemiluminescence of luminol [108]. The decreased electrochemiluminescent intensity was linear with dopamine concentration in the range of 5.0×10^{-8}–1.0×10^{-5} mol/l with a detection limit of 30 nmol/l and a RSD of 1.9% ($n=11$) for 1.0×10^{-6} mol/l dopamine and the method was applied in pharmaceutical injections.

8. Flow injection analysis/High performance liquid chromatography

The combination of flow injection analysis with high performance liquid chromatography still forms a powerful combination in the determination of catecholamines. A system was presented for the determination of catecholamines in human urine using the combination of flow injection analysis coupled to high performance liquid chromatography with chemiluminescence detection [109]. The detection method was based on the inhibition effect of catecholamines on the CL reaction of luminal with iodine in alkaline medium. The proposed FI method allows the determination of catecholamines in pharmaceutical preparations for the purpose of drug quality control. The HPLC procedure was successfully applied for the determination of catecholamines in human urine after solid-phase extraction. In a simple run time catacholamines could be determined in 20 min. A system was also developed for the simultaneous determination of free catecholamines in urine by using tandem affinity/ion-pair chromatography with amperometric detection and FIA with UV–vis spectrophotometric detection [110]. The ability of the total system to monitor the variety of analytes made the HPLC/FIA system an attractive option for use in monitoring urinary compounds.

9. Conclusions

The importance and successful implementation of flow-injection analysis systems with different detection devices and other related techniques for the in vitro and in vivo determination of dopamine as neurotransmitter forms the heart and back bone of the diagnosis and successful treatment of neurological disorders, such as schizophrenia and Parkinson's disease in humans. The variety of detection and related systems coupled to FIA include UV–vis spectrophotometric measurements, ion selective electrodes, amperometry, fast-scan cyclic voltammetry, micro-chip-based microfluidic systems, chemiluminescence and electrochemiluminescence and high performance liquid chromatography. It is clear that the electrochemical detectors formed by far the choice for in depth studies of in vitro and in vivo determination of dopamine in the human brain with HPLC still the choice for separations. With the new developments in quantum dots, the field is open for further studies in this regard.

Acknowledgments

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