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A steady-state and flow-through cell for screen-printed eight-electrode arrays

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Abstract

An electrochemical cell has been developed enabling amperometric steady-state- and flow-injection measurements with screen-printed arrays consisting of eight working electrodes ($\emptyset = 1 \text{ mm}$) arranged radially around a printed Ag/AgCl reference electrode in the centre. The cell contained a rotator, providing similar hydrodynamics over all the working electrodes in the array, which was manually centered under the rotator. The reproducibility of steady-state measurements with eight-electrode platinum or gold arrays in this cell was studied by measuring and comparing currents from ferricyanide reduction at each electrode in the array. It was found that the relative standard deviation (R.S.D.) for the currents at different electrodes on one array was below 5%. Similar R.S.D. was found if measurements were compared between several arrays. This indicates that manual insertion/positioning of the eight-electrode array in the cell and hydrodynamics at the electrodes provided measurement reproducibility similar to the reproducibility of manufacturing eight-electrode platinum or gold arrays by screen-printing. A comparative study was performed between screen-printed and through mask sprayed carbon arrays. It was found that the reproducibility of the sprayed arrays was similar to that of the platinum or gold screen-printed arrays, with R.S.D. values below 6% regarding the variation between electrodes within the same array and the variation between different arrays. To enable flow-injection measurements, a tube (0.4 mm inner diameter) was inserted into a hole drilled through the centre of the steady-state cell rotator. This construction made it possible to inject the solution into the cell through the tube (not rotating), while the rotator was spinning over the eight-electrode array. It was found that this combination of flow-injection and mixing by a rotator provided a uniform current response over the array electrodes and that, at optimum conditions, the R.S.D. values between the eight electrodes in the array were nearly the same as in case of the steady-state measurements, i.e., below 5%.

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1. Introduction

Compared to single electrodes, arrays have the advantage of testing multiples of analytes simultaneously. This is one of the reasons why the research on electrochemical arrays has become of recent interest (for a review see [1]). Application

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examples can be found in food, environmental or clinical analytical chemistry, where arrays offer advantages such as fast and simple measurements directly in-field without any sample pretreatment.

A special area of array science comprises the electronic tongue built from poorly selective sensors responding to a number of different chemicals or classes of chemicals [2–5]. The idea behind the electronic tongue is that every sample has its own unique fingerprint on the array and that it is thereby possible to classify complex matrices using pattern recognition software programs [6]. Multivariate sample differentia-

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tion may generally be enhanced if the sensor collection contains as much chemical diversity as possible, so that the array responds to the largest possible cross-section of analytes [7]. Hence, a good choice for making electronic tongues is to use amperometric array systems, since the responses and the selectivity of the electrodes in the array can easily be varied by applying different potentials at individual electrodes, varying electrode material, modifying electrodes in different ways, or using a combination of these approaches.

A trend in the development of amperometric arrays is the movement towards miniaturized systems. Several advantages are obtained by microarray systems such as increased mass-transport due to radial diffusion (resulting in a faster response at the transducer), reduced double-layer capacitance due to smaller electrode areas (the signal to noise ratio is increased), and reduced ohmic drop [8,9]. On the other hand, the current output is lower; and parallel connection of microelectrodes might be needed to receive a detectable current response [10,11]. Another attractive aspect with microarray systems is the reduced effect from fluctuations in the flow rate compared to macroscopic constructions [9]. Several examples of amperometric microarray flow systems have been reported [12,13].

Despite the positive properties attributable to amperometric microarray systems, there are situations where macroarrays can be more favorable. Generally, macro systems are more robust against negative effects such as contamination (dust particles, pollutants in the matrix, impure enzymes, etc.) and chemical cross-talk between the sensors [14]. The directed immobilization of functional proteins on individual microscopic regions is still a challenge [9]. Thus, a macroarray system can be a better choice if the purpose is to create biosensor arrays, especially at the initial stages of their development. For use of biosensor arrays as bioelectronic tongues, a slow response (i.e., time dependence of the response) obtained in amperometric macro flow systems could be utilized as an additional factor to discriminate samples with pattern recognition methods. The differences of reaction kinetics are often reflected in the shape of the response curves [15,16].

A number of different macroarray geometries for flow systems have been described in the literature. One of the most popular choices has been to construct cells based on serial arrangements of the working electrodes [17–20]. Even though such systems offer advantages of equal flow and sensitivity over the entire array, several drawbacks have been reported. Difficulties were observed in maintaining potentiostatic control over electrodes in a thin-layer serial glassy carbon array due to ohmic drop [18]. A continuous increase of dispersion from electrode to electrode in an array when the sample was pumped through the flow channel was demonstrated for an array of eight serially distributed potentiometric sensors [19]. Another aspect is that chemical cross-talk can occur when the product from an upstream electrode causes non-specific responses on a downstream electrode [14]. From our experience, these drawbacks are especially limiting at the initial development stages of biosensor arrays when efforts are made to understand the performance of each single biosensor in the array.

Radial positioning of electrodes in an array can reduce some of these disadvantages but can instead impose difficulty in providing equal hydrodynamics over the electrodes in the array [21-23]. Hoogvliet et al. described already in 1991 [21] a flow-through amperometric system with an array holder of 16 carbon paste electrodes positioned radially at a distance of 4 mm from the centre containing the solution injection port. The sensitivity due to decreased mass transfer was approximately one-third of the sensitivity that could have been obtained with one working electrode of the same dimension placed in the middle of the cell [24]. However, no chemical cross-talk was observed. The R.S.D. of the average response was 4.5%, which was of the same level of precision due to regeneration of a fresh carbon surface for an individual electrode. Fielden and McCreedy constructed a similar system for eight glassy carbon electrodes [22] and also evaluated cell characteristics by varying the separation distance between the inlet jet and the planar array surface [18]. The system showed typical thin-layer behavior at low distances (<1 mm), where small changes in the distance had obvious effects on the electrode sensitivity. At large distances (>6 mm), the system acted as a wall-jet cell. Chen et al. have described a large-volume wall-jet cell for an array of four radially distributed carbon paste electrodes modified with different metal oxide catalysts [23]. The system was used for quantitative determination of two and three component mixtures of carbohydrates and amino acids by pattern recognition methods. All described systems have technically realized the goal of strictly fixing the injection port to the radial distribution of the electrodes, i.e., the hardware construction is made in a way that the position of the electrodes versus the position of the injection port cannot be changed. The reviewed technical solutions, however, are not suitable for working with disposable radial electrode arrays, especially, in cases where one wishes to work with different screen-printed electrodes (e.g., radial distribution of 4, 8, or 16 electrodes) and use the same amperometric cell. Keeping this in mind, it should be emphasized that the work presented here describes simple and highly practical design of an electrochemical cell for working with different arrays consisting of radially distributed electrodes.

The advantage of using disposable arrays with integrated working and reference electrodes is that they can be mass-produced at a low cost by screen-printing technology [25–29]. The electrodes are formed by squeezing an ink containing the electrode material through a mask onto a substrate (usually made of a polymeric or ceramic material). The possibility to easy vary the ink composition and the ability to deposit materials in several layers has made screenprinting technology popular for construction of thick-film biosensors [25,27]. To our knowledge, an electrode array system consisting of radially distributed screen-printed electrodes providing equal steady-state- or flow-injection current signals from each electrode in the array has not yet been described. In order to succeed with this, the analytical cell must provide equal hydrodynamics at each of the electrodes and the electrodes should be reproducibly applied on the array (position, size, surface properties). Two ways to improve the overall precision for measurement with radial screen-printed eight-electrode arrays are demonstrated in this article. First, a cell enabling both steady-state- and flow-injection measurements that generate equal hydrodynamics over all electrodes in the array has been constructed. Secondly, as an alternative to screen-printing a carbon ink on a screen-printed platinum array, reproducible carbon-based arrays have been developed by spraying a carbon ink through a mask on top of a screen-printed platinum array. The work is directed towards developing a technical basis for construction of bioelectronic tongues. Initial data on carbon-based arrays modified with horseradish peroxidase (HRP) are presented.

2. Experimental

2.1. Chemicals

Hydrogen peroxide (30%), K₃Fe(CN)₆, K₄Fe(CN)₆, catechol, acetone, cyclohexanone and the buffer chemicals were purchased from Merck (Darmstadt, Germany). Cellulose acetate (\sim 40% acetyl), graphite powder (1–2 micron, synthetic), and HRP were from Sigma-Aldrich (Steinheim, Germany). All aqueous solutions were prepared using water purified with a Milli-Q system (Millipore, Bedford, USA).

2.2. Electrodes

Screen-printed arrays (product numbers AC9.W1.R1, AC9.W2.R1, AC9.W4.R1) consisting of eight working electrodes (each electrode with a diameter of 1 mm), arranged radially around a printed Ag/AgCl reference electrode, were from BVT Technologies a.s. (Brno, Czech Republic, http://www.bvt.cz), see Fig. 1C. The working electrodes were printed gold, platinum or carbon paste DP7101 (Dupont, USA) on platinum, respectively.

The sprayed carbon arrays were prepared by spraying carbon ink on top of screen-printed platinum arrays. The carbon ink consisted of a graphite mixture of 5.2 g of graphite (1–2 micron, synthetic from Sigma-Aldrich), 0.5 g of cellulose acetate, 150 ml of acetone, and 10 ml of cyclohexanone. Graphite mixture of 1 ml was then sprayed through a brass mask, which contained holes at positions matching each individual working electrode on the eight-electrode array.

2.3. Preparation of HRP-modified carbon-based arrays

Arrays of screen-printed and sprayed carbon were modified with HRP. For this, $1 \mu l$ aliquot of HRP solution (5 mg/ml) was added on top of each working electrode. The enzyme in solution was allowed to adsorb for 20 min under a glass beaker. Before use, the array was carefully rinsed with Milli-Q water.

2.4. The amperometric eight-electrode steady-state cell

A cell permitting steady-state measurements was constructed from Plexiglas to fit the eight-electrode arrays, see Fig. 1. The screen-printed array (a) was inserted through a rectangular hole (b) in the beaker and adjusted manually so that the array ring was centered in the hole (c) at the bottom in the beaker. The array was tightened in the cell by a Plexiglas screw pressing the array electrode towards the round edge of the hole, which was modified with silicon resin to prevent leakage of the solution. A rotator (d), with the same diameter as the hole (c), was placed at the desired distance (in the range from 0.16 to 5 mm) over the array surface. The design of the cell has been patented [30]. The eight working electrodes were independently controlled by an 8-channel potentiostat (two electrode system with working electrodes and a Ag/AgCl reference/counter electrode printed on the array) and data were collected with the software program Intels 1.5. The potentiostat and the software (homemade, Prof. J. Kulys, Laboratory of Enzyme Chemistry, Institute of Biochemistry, Vilnius, Lithuania) were specifically constructed for amperometric measurements with eightelectrode arrays.

2.5. The amperometric eight-electrode flow-through cell

A flow-injection cell was constructed on the basis of the electrochemical steady-state cell (Fig. 1). The inlet of the flow-injection cell was constructed by drilling a hole through the middle of the rotator and inserting a stainless steel tube (0.4 mm inner diameter), which remained still while spinning the rotator. The outlet flow was constructed via a needle glued on the inner wall of the cell. Otherwise, the conditions were the same as in the steady-state cell shown in Fig. 1.

2.6. Procedures

The reproducibility of the arrays and the performance of the amperometric steady-state cell were evaluated by the reduction of $K_3Fe(CN)_6$ in acetate buffer (50 mM, pH 5) containing 0.1 M KCl at -50 mV versus Ag/AgCl. For steady-state measurements, different volumes of 10 mM $K_3Fe(CN)_6$ stock solution were added into the cell, previously filled with 10 ml of acetate buffer. During steady-state amperometric measurements, the ferricyanide concentration in the cell was in the range from 0.02 to 1 mM.

To evaluate HRP-modified carbon-based arrays, the cell was filled with 100μ M solution of H_2O_2 in phosphate buffer (20 mM, pH 7) containing 0.1 M KCl. A baseline current was observed due to direct electron transfer between HRP and the electrodes [31]. After stabilization of the baseline current,

A. The 8-electrode steady-state cell



B. Cross section view of the steady-state cell



C. The 8-electrode array construction



Fig. 1. (A) Photograph of the amperometric steady-state cell made of Plexiglas. The array (a) is inserted into a rectangular hole (b) and the electrode surfaces are centered manually in the hole (c) at the bottom of the cell. The array is tensed in the cell via a screw, where some silicon is applied under the bottom hole (c) to keep the contact between the array surface and the cell watertight. An adjustable rotator (d) mixes the solution tightly over the array surface. (B) Cross-section view of the steady-state cell. (C) The radial eight-electrode array construction.

catechol was added to the cell. Steady-state measurements were carried out at different concentrations of catechol in the range from 5 to $200 \,\mu$ M. In this case, current responses at HRP-modified electrodes are known to be due to mediated electron transfer between HRP and the electrode surface [32].

The arrays were also characterized with cyclic voltammetry using a CV 50 W potentiostat (BAS, Bioanalytical Systems, West Lafayette, IN, USA), with a three-electrode configuration using an external saturated calomel reference electrode (SCE), and a platinum wire as a counter electrode. One electrode at a time in the array served as a working electrode in these CV measurements. A mixture of 5 mM K₃Fe(CN)₆ and 5 mM K₄Fe(CN)₆ was used. The potential was varied between -100 and 500 mV (versus SCE) at a scan rate of 20 mV/s.

The amperometric flow-injection cell experiments were performed on a platinum array by injecting 0.2 mM K₃Fe(CN)₆ dissolved in the flow carrier of acetate buffer (50 mM, pH 5) containing 0.1 M KCl, at an electrode potential of -50 mV versus Ag/AgCl. A peristaltic pump (Gilson minipuls 2) transported the carrier buffer in and out from the cell. The sample was introduced through a 200 µl injection loop connected to a Rheodyne (Berkeley, CA, USA) six-port injection valve.

3. Results and discussion

3.1. Evaluation of the steady-state cell

To provide equal hydrodynamic conditions on an array with eight screen-printed electrodes, a special steady-state cell was constructed. The cell allowed manual centering of the array directly under a rotator, which provided mixing of the solution over the eight electrodes. An example of amperometric (steady-state current) responses from each of the eight working electrodes in a screen-printed gold array is presented in Fig. 2. To evaluate the performance of the cell, the following dependencies were investigated: (a) the dependence of electrode current as a function of the distance between the rotator and the array, and (b) the dependence of current as a function of the angular frequency of the rotator. In all experiments for evaluation of the steady-state cell, the reduction current for 0.1 mM ferricyanide was measured on an eight-electrode platinum array. The distance between the rotator and the array surface was varied, while the angular frequency was kept constant at 15 Hz. As can be seen in Fig. 3, the steady-state current exhibited a slight dependence on the distance between the rotator and the electrode array. The difference between the maximal and the minimal steady-state current was less than 20% for distances from 0.16 to 4 mm.



Fig. 2. Steady-state currents due to reduction of 0.02, 0.05, and 0.1 mM K_3 Fe(CN)₆, in acetate buffer (50 mM, pH 5) containing 0.1 M KCl, on an eight-electrode gold array at -50 mV vs. Ag/AgCl. The angular frequency of the rotator was 15 Hz and the distance between the rotator and the array surface was 1.7 mm.



Fig. 3. Amperometric cell steady-state measurements with 0.100 mM K₃Fe(CN)₆ in acetate buffer (50 mM, pH 5) containing 0.1 M KCl at -50 mV vs. Ag/AgCl. The plot shows how the current output varies with the distance between the rotator and the array surface. The angular frequency of the rotator was kept constant (15 Hz).

As seen in the insert in Fig. 3, the current signals became noisier at greater distances.

To study the effect of angular frequency of the rotator on the steady-state currents, the rotator was fixed at 1.7 mm, while the angular frequency was varied between 2 and 20 Hz. From the results, summarized in Fig. 4, it can be seen that the current is higher at higher angular frequency. The current at 20 Hz is about three times that at 2 Hz. The increase of the current is obviously caused by higher flux of ferricyanide to



Fig. 4. Amperometric cell steady-state measurements with 0.100 mM K₃Fe(CN)₆ in acetate buffer (50 mM, pH 5) containing 0.1 M KCl at -50 mV vs. Ag/AgCl. The plot shows how the current output varies with the angular frequency of the rotator. The distance between the rotator and the array surface was kept constant (1.7 mm).

the electrodes due to decrease of the hydrodynamic boundary layer (and thus the diffusion layer) at higher angular frequency of the rotator. It can be noticed from Fig. 4 that, when the angular frequency exceeds 10 Hz, a step occurs in the dependence between current versus angular frequency. Similarly, some step could be recognized when the distance exceeds 1 mm in previously discussed current versus distance dependence (Fig. 3). Both these irregularities could probably be assigned to the edge effect caused by construction of the cell (i.e., a circular edge of the hole in the bottom of the electrochemical cell, where the array is placed for having contact with the solution in the cell) and thus, we believe do not present a general phenomenon.

From the experiments described above, it can be concluded that a high current output combined with low noise and low spread between the responses (i.e, low R.S.D.) from the eight array electrodes is obtained at a rotator distance of ~ 1 mm and an angular frequency of ~ 15 Hz. Therefore, these parameters were considered optimal for direct electrochemical measurements with these arrays. However, for the electrodes modified with enzymes, it is likely that enzyme kinetics (e.g., inhibition effects) and different thickness of the sensing layers will require different hydrodynamic conditions for optimal performance.

To test the reproducibility of the measurements provided by the cell, the following experiments were performed. The rotator was lifted from its position (in this case, 1.7 mm above the array surface) and then returned to this position 10 times, and between each movement a current was recorded. The variation of the response for each array electrode over these measurements, noted as the relative standard deviation (R.S.D.), was between 1.5 and 1.7%. Next, the variation due to manual adjustment of the array in the cell was evaluated by removing the array and reinserting it into the cell 10 times. The R.S.D. values were found to be slightly higher for the currents at electrodes on a single array, spanning from 2.7 to 4.8%, which indicates that the precision of centering the array under the rotator is important. The R.S.D. due to manual positioning of the array in the cell is still fairly low and can be considered acceptable to use the cell to investigate the properties of radial electrochemical arrays.

3.2. Evaluation of eight-electrode arrays by steady-state measurements

For an ideal cell, we expected that the measurement variations due to unequal (not reproducible) hydrodynamics at the electrodes would be lower than those caused by the reproducibility of array production. To test this hypothesis, amperometric measurements were conducted with a number of eight-electrode arrays including screen-printed platinum, gold, carbon, and carbon ink arrays. Variations between electrodes both within the same array and between different arrays were evaluated by comparing the slope values from the calibration graphs constructed from current versus concentration measurements (from raw data similar to

Table 1 Reproducibility of amperometric steady-state measurements with different eight-electrode arrays

Array type	Slope $(\mu A m M^{-1})$	R.S.D. (%) $n = 8$	Mean slope $(\mu A \mu M^{-1})$	R.S.D. (%) n=3
Gold	2.705	1.8	2.784	6.4
Gold	2.988	2.3		
Gold	2.659	4.6		
Platinum	2.974	1.6	2.993	0.6
Platinum	3.012	3.7		
Platinum	2.994	3.7		
Printed DP 7101	1.950	12	3.301	35
Printed DP 7101	3.990	14		
Printed DP 7101	3.962	17		
Sprayed carbon	2.260	4.6	2.351	5.7
Sprayed carbon	2.506	3.5		
Sprayed carbon	2.287	2.3		

Variation between electrodes within the same array (column 3) and variation between different arrays (column 5) are calculated as R.S.D.s of the slopes of ferricyanide calibration graphs (0.02–1 mM).

those in Fig. 2). The results are summarized in Table 1. Variations between electrodes within the same array for platinum (R.S.D. 1.8-4.6%) and gold (R.S.D. 1.6-3.7%) were found to be of the same magnitude if compared to variations from the cell reproducibility test when the array was taken out and reinserted into the cell (R.S.D. 2.7-4.8%). These results indicate that R.S.D. values obtained with screen-printed platinum or gold arrays are not caused by the precision of the array production, but rather by different hydrodynamics at the electrodes in the array. The R.S.D. values for the screenprinted carbon arrays were, however, noticeably higher, i.e., 12-17%. Sprayed carbon arrays using the homemade ink resulted in a much better reproducibility, with R.S.D.s of 2.3-4.6%, which is practically the same as that obtained for gold and platinum. Comparison of the variation between different arrays showed the same trend, the R.S.D. for screenprinted carbon were much higher (R.S.D. 35%) than for sprayed carbon arrays (R.S.D. 5.7%). Thus, it can be concluded that spraying the paste on the array produces highly reproducible carbon electrodes. The high R.S.D. for screenprinted carbon electrodes may probably be due to a nonhomogenous distribution of binding polymer and carbon [33]. The same conclusion could also be drawn from CV recordings, where the separation of the oxidation and the reduction peak ($\Delta E_{\rm p}$) was found to be much less reproducible for the screen-printed carbon electrodes. The ΔE_p values (and their R.S.D.s) in one array were as follows: 91 mV (1.4%) for gold electrodes, 79 mV (3.2%) for platinum, 114 mV (11.7%) for screen-printed carbon, and 86 mV (1.4%) for sprayed carbon. Only one array was tested of each electrode type. In addition to the poor reproducibility, the screenprinted carbon electrodes also showed reduced reversibility for the ferri-/ferrocyanide reaction, which might indicate limited suitability of these electrodes for the development of biosensors relying on fast heterogeneous electron transfer processes [33].



Fig. 5. Calibration plots for catechol in phosphate buffer (20 mM, pH 7) containing 0.1 M KCl at a horseradish peroxidase-modified (a) screen-printed DP 7101 carbon array and (b) sprayed homemade carbon ink array. The enzyme was activated by 0.100 mM H_2O_2 before adding catechol to the cell. Applied potential: -50 mV vs. Ag/AgCl. Measurements were performed by the steady-state cell with a rotator angular frequency of 15 Hz and a distance between the rotator and the array surface of 1.7 mm.

3.3. Enzyme-modified carbon-based arrays evaluated by steady-state measurements

To investigate the use of the cell and the screen-printed electrodes for array biosensors, carbon arrays were modified with horseradish peroxidase (HRP) by simple adsorption. The main purpose of this experiment was to obtain an initial indication for the reproducibility of the amperometric responses of enzyme-modified arrays. It is known that simple phenolic compounds including catechol function as mediators for the bioelectrocatalytic reduction of hydrogen peroxide at peroxidase-modified electrodes [32,34]. In Fig. 5, calibration curves for catechol recorded for two types of carbon arrays show that the variation between electrodes within the same array in general is lower for the sprayed carbon array (i.e., the electrode giving the highest signal in Fig. 5b can be viewed as an outlier, statistically supported by a Qtest) and the linear range is significantly better than for the screen-printed carbon array. Since the sprayed carbon electrodes were highly reproducible for the ferricyanide redox reaction (as judged from CVs), the higher spread of the calibration curves for catechol recorded with HRP-modified electrodes must be explained by a dependence of the biosensor signal on factors other than the surface properties of the carbon electrodes, e.g., inhomogeneous enzyme distribution on the surface, manual application of the enzyme solution, etc. It is well known that reproducible fabrication of biosensors can be tricky and much more knowledge- and technologydemanding compared to bare electrodes. It is expected that the same should be true for the development of biosensor arrays versus bare electrode arrays.

Table 2 R.S.D. values (%) for peak current responses of ferricyanide (0.2 mM in acetate buffer) reduction at eight Pt electrodes on an array measured with the flow-through cell

Angular frequency (Hz)	Hz) Distance (mm)						
	0.16	0.20	0.30	0.36	0.40	0.50	
Rotation off	18	21	17	13	15	35	
12	30	18	18	13	18	13	
24	24	24	13	13	6.9	16	
35	7.0	5.5	35	31	25	27	
41	4.6	5.4	42	38	27	No peal	
47	5.8	8.0	45	40	31	No peal	

The angular frequency of the rotator as well as the distance between the rotator/injection port and the array were varied. "No peak" means that it was impossible to observe a clear peak response due to extreme stirring effects.

3.4. Evaluation of the array flow-through cell

The flow-through cell was developed by simply incorporating a flow channel through the rotator of the steady-state cell. To find the optimal conditions for peak shape and signal reproducibility, amperometric measurements were performed at different flow rates, distances between injection port and array, and angular frequency of the rotator. It was found that a relatively high flow rate was needed as flow rates lower than 3.0 ml/min resulted in tailing flow-injection peaks as well as response times exceeding 6 min. Therefore, a flow of 3.7 ml/min was used for subsequent experiments.

The R.S.D. values obtained for different distances between the rotator and the array at different angular frequencies are summarized in Table 2. The spread between the responses for the electrodes within the array was the lowest when the distance was low and the angular frequency was high. The best result (R.S.D. = 4.6%) occurred at an angular frequency of 41 Hz and a distance of 0.16 mm (Table 2 and Fig. 6a). The R.S.D. value under these conditions was practically the same as for the steady-state cell (<5%). Earlier, Hoogyliet et al [21] reported similar reproducibility for radially distributed carbon paste electrodes. In addition, the eight peak



Fig. 6. Flow cell reduction responses for $0.2 \text{ mM } \text{K}_3\text{Fe}(\text{CN})_6$ in acetate buffer (50 mM, pH 5) containing 0.1 M KCl at -50 mV vs. Ag/AgCl. The angular frequency of the rotator and the distance between the outflow and the array were (a) 41 Hz, 0.16 mm, (b) 0 Hz, 0.16 mm and (c) 41 Hz, 0.30 mm.

maxima (Fig. 6a) coincided nicely at the optimized condition, indicating that the flow-through cell construction provides similar hydrodynamic conditions at each electrode in the array. This was supported by the dispersion factor, which was found to be equal to 2.7 for each electrode in the array. The dispersion factor was determined as the ratio between the steady-state current and the flow-injection peak current. For comparison, flow-injection peaks recorded at other measurement conditions can be seen in Fig. 6b and c. It is obvious that decreased angular frequency and an increased distance between the injection port and the array have negative effects on the reproducibility.

The reproducibility of the flow-through cell was evaluated for variations due to manual adjustment of the array into the cell (the array was taken out and reinserted into the cell 10 times). The R.S.D. for each array electrode for these 10 measurements was between 3.1 and 10.3%. The system likely works as a thin-layer cell [18] (0.16 mm between the injection port and the array electrode) and a small deviation in positioning translates into higher distortion of the peak current response compared to steady-state measurements. This indicates that, in general, a more precise positioning of the array electrodes in the flow-through cell is required to achieve reproducibility of peak responses and their shapes.

4. Conclusions

An amperometric cell enabling steady-state and flowinjection measurements has been constructed for screenprinted arrays consisting of eight electrodes distributed radially. The critical factor, limiting the reproducibility of the measurements, seems to be the manual positioning of the array into the cell. However, R.S.D.s below 5% are achievable. We regard this reproducibility as sufficient for development of biosensor arrays as well as for investigation of electrochemical array systems consisting of radially distributed screen-printed electrodes. The system reliability should make it possible to use the arrays for real-time analysis of multicomponent mixtures.

For many biosensors, carbon-based materials are the most suitable; but for an array of electrodes modified with different enzymes, other electrode materials also may be needed. We have demonstrated that spraying of carbon ink can be considered an alternative to screen-printing, especially, in cases, where the development requires both metals (e.g., Pt, Au) and carbon electrodes together on a single array.

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