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Multichannel separation device with parallel electrochemical detection

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ABSTRACT

A device with four parallel channels was designed and manufactured by 3D printing in titanium. A simple experimental setup allowed splitting of the mobile phase in four parallel streams, such that a single sample could be analysed four times simultaneously. The four capillary channels were filled with a monolithic stationary phase, prepared using a zwitterionic functional monomer in combination with various dimethacrylate cross-linkers. The resulting stationary phases were applicable in both reversed-phase and hydrophilic-interaction retention mechanisms. The mobile-phase composition was optimized by means of a window diagram so as to obtain the highest possible resolution of dopamine precursors and metabolites on all columns. Miniaturized electrochemical detectors with carbon fibres as working electrodes and silver micro-wires as reference electrodes were integrated in the device at the end of each column. Experimental separations were successfully compared with those predicted by a three-parameter retention model. Finally, dopamine was determined in human urine to further confirm applicability of the developed device.

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1. Introduction

Several protocols are currently being used in chromatography to increase analytical throughput and to speed up a method development. A widespread approach is to use ultra-high-pressure liquid chromatography, using sub-2- μm particles [1]. Another approach relies on column-switching techniques to allow samples to be analysed on various columns simultaneously [2–4]. Separated compounds are then monitored using one detector or a combination of several detectors in series (e.g. UV and MS) [5]. Another possibility to increase analytical throughput is to apply multiple parallel channels. However, such an experimental setup requires multiple simultaneously operating detectors.

For example, laser-induced fluorescence [6] and UV detection [7] have been used in combination with multi-channel capillary electrophoresis and two-dimensional liquid chromatography, respectively.

Hence, miniaturization and multiplexing of detection systems is crucial for the development of multichannel analytical devices providing parallel analysis and detection of samples in capillary format. Ryvolová et al. developed a “3-in-1 detector” combining

photometric, fluorimetric, and contactless conductivity on-capillary detection [8]. Microchip non-aqueous electrophoresis coupled with contactless conductivity detection has been used for high performance separation of quaternary amines [9]. A deep UV-LED with a peak emission wavelength of 235 nm for photometric detection and its application in an on-capillary detector have been also presented [10]. Further, 3D printing technology can be used to fabricate a fluorescence detector for capillary separations [11]. An electrochemical detection is very powerful method, especially when coupled to microchip multichannel devices [12–15] or paper-based electrochemiluminescence immunodevices [16–18]. Recently, we have used unsized carbon fibres together with a silver microwire to prepare a miniaturized electrochemical detector, integrated within a polymer-based monolithic capillary column [19,20]. Stable and robust chromatographic analyses and multiple-pulse amperometric detection of neurotransmitters were achieved. This integrated device has also been used successfully to determine dopamine in a human urine.

Additive manufacturing (i.e., 3D printing) is currently drawing much interest from the research community especially due to its ability to make complex structures with high resolution. At first, desired design is digitally built. Then, fabrication a complete microfluidic device proceeds in a single step by adding selected materials. 3D printed microfluidic chips are being applied in both chemistry and biology. Additionally, sensors and actuators can be inte-

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grated with the microfluidics during printing allowing preparation of truly multifunctional devices [21–24].

In HPLC, column efficiency and selectivity are predominantly controlled by the structure and surface chemistry of the stationary phase. Silica-gel is a popular material used for the preparation of particulate stationary phases [25]. Alternatively, polymer-based monolithic materials [26] are being used as complementary stationary phases, allowing simple tuning of surface chemistry by either selecting desired functional monomers [27] or tailoring post-polymerization surface modification [28,29]. Moreover, polymer monoliths can be easily prepared in almost any size and shape, facilitating their application in miniaturized analytical systems. The combination of simple selectivity control and easy miniaturization make polymer monoliths attractive for the development and fabrication of a multichannel analytical device that provides parallel analysis and detection of complex samples.

Dopamine is one of the most-important neurotransmitters and undesired changes in its metabolism may result in serious illnesses, such as depression, schizophrenia, Parkinson disease, and tumors [30,31]. Electrochemical detection is well-suited for the analysis of neurotransmitters and can be miniaturized and parallelized.

In this work we aimed to prepare a miniaturized separation device, combining various monolithic stationary phases with an electrochemical detection to allow parallel simultaneous analysis of a single sample of dopamine precursors and metabolites.

Selectivity differences of prepared monolithic stationary phases provide sample information that would be difficult to obtain on a single column, unless multiple injections and/or experimental conditions are involved. Additionally, utilization of both monolithic stationary phases and electrochemical detection enables development of compact miniaturized instrumentation for future fabrication of portable analytical systems applicable for diagnostics of dopamine-metabolism-related diseases and for treatment monitoring.

2. Experimental part

2.1. Chemicals and materials

3-(trimethoxysilyl) propyl methacrylate, sodium hydroxide, hydrochloric acid, trifluoroacetic acid (TFA), 1,4-butanediol, and 2,2'-azobisisobutyronitrile were purchased from Fluka (Buchs, Switzerland). N,N-dimethyl-N-metacryloxyethyl-N-(3-sulfopropyl) ammonium betaine, tetramethylene dimethacrylate, hexamethylene dimethacrylate, dioxyethylene dimethacrylate, bisphenol A glycerolate dimethacrylate, 1-propanol, acetone, epinephrine

hydrochloride, dopamine hydrochloride, norepinephrine hydrochloride, homovanillic acid, 3,4-dihydroxyphenyl acetic acid, 3,4-dihydroxy-L-phenylalanine, 2-phenylethylamin hydrochloride, L-phenylalanine, tyramine, and tyrosine were obtained from Sigma-Aldrich (St. Louis, MI, USA). Acetonitrile for gradient HPLC (Merck, Darmstadt, Germany) and redistilled deionized water were used for preparing the sample and the mobile phase. Polyimide-coated 320- μm i.d. fused-silica capillaries were purchased from Agilent (Palo Alto, CA, USA). Carbon fiber with a diameter of 7 μm and silver microwire with a diameter of 25 μm were obtained from Goodfellow Cambridge (Huntingdon, United Kingdom). Conductive silver paint was purchased from Ted Pella (Redding, CA, USA) and ceramic slides from Coors Ceramics (Golden, CO, USA).

2.2. Instrumentation

A modular micro liquid chromatograph was assembled from a Shimadzu LC10ADvp pump (Shimadzu, Kyoto, Japan), a micro-valve injector with an internal 60-nl loop for direct injection on capillary columns or a valve with a 200-nL external sampling loop in case of a mobile-phase split-flow arrangement (both Valco, Houston, USA), controlled using an electronic actuator. A restrictor capillary was inserted as a mobile-phase flow splitter before the injector, in case only a single column was being characterized. A Sapphire capillary detector (ECOM, Prague, Czech Republic) was used as a UV detector. In electrochemical detection, current responses in multiple pulse mode were recorded with a two-electrode arrangement using PalmSens and EmStat4WE with PStTrace software (PalmSens, Houten, The Netherlands). Multiple-pulse amperometry was employed using the sequence of potentials +1.0V for 0.4 s (measuring of current), -1.0V for 0.4s and 0V for 0.2 s. Here, very short time sequences of detection potential alternate with a potential that is used for cleaning the electrodes [19]. Fused-silica capillary monolithic columns were fitted directly into the body of a micro-valve injector or, in case of flow-splitting, in the second level T-Piece. An arrangement of flow-splitting T-Pieces is schematically shown in Fig. 1.

2.3. Titanium 3D-printed device

Titanium, which is a strong, lightweight, and corrosion-resistant material, allows printing by sintering titanium powder together with a laser [32]. Titanium devices show high strength-to-weight ratio and are sterilizable and biocompatible. Fig. 1 shows the spatial orientation of a four-channel 3D-printed device with inlets of the sample/mobile phase, arrangement of flow splitters, and outlets where microelectrodes were attached. The channel array was

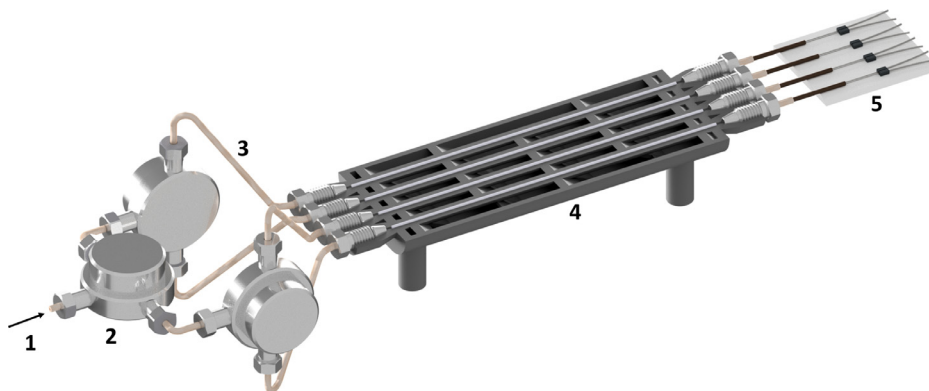


Fig. 1. Experimental setup used in simultaneous parallel analysis with 3D printed titanium device utilizing monolithic stationary phases and an integrated electrochemical detection. 1 – sample and mobile phase inlet, 2 – flow-splitting T-piece, 3 – fused-silica capillary (80 \times 0.025 mm), 4 – 3D-printed four-channel device with monolithic stationary phases, 5 – fused-silica capillary with an integrated microelectrodes attached to a ceramic support slab. Dimensions of channel 100 \times 0.8 mm.

printed using an SLM 50 Realiser (Borchen, Germany) in the Ti-6Al-4V alloy, with the dimensions of each channel being 100×0.8 mm. 10–32 HPLC fitting threads were tapped onto the inlet and outlet slots. Prior to the creation of monolith in titanium capillaries (i.e. as the last step in the fabrication process), the surface was oxidized to create a reactive layer of titanium oxide. Therefore, the device was then placed in an ashing furnace (AAF 11/3, Carbolite, Carbolite Gero Ltd, United Kingdom) at 600 °C for 6 h to oxidize the channels' surfaces for surface attachment of the polymer monolith.

2.4. Preparation of monolithic stationary phases

Monolithic capillary columns were prepared according to protocols published previously [33]. The inner wall of the capillary surface was first modified with 3-(trimethoxysilyl) propyl methacrylate prior the polymerization reaction. Then, the monolith was prepared in the capillary using an *in-situ* radical reaction of a polymerization mixture containing 0.20 g of N,N-dimethyl-N-methacryloxyethyl-N-(3-sulfopropyl) ammonium betaine and 0.15 g of crosslinking dimethacrylate monomer, dissolved in a porogenic solvent mixture consisting of 0.25 g 1,4-butanediol, 0.25 g 1-propanol, and 0.15 g water. Finally, 0.0035 g of azobisisobutyronitrile initiator was added to the polymerization mixture. The prepared polymerization mixture was sonicated for 10 min and filled in the vinylized capillaries with internal diameter of 0.32 mm and length of 150 to 180 mm. An air plug (2 mm) was left at the end of the capillary to allow space for the integration of microelectrodes. Both ends of the capillary were sealed with stoppers and the capillary was placed in a circulated air oven, where polymerization reaction proceeded at 60 °C for 20 h. After that, the monolithic capillary columns were flushed first with acetonitrile, followed by mobile phase. The preparation of the monolithic stationary phases inside titanium 3D-printed device followed the same procedure as in case of capillary columns.

2.5. Integration of an electrochemical detection

A detailed description of the integration of an electrochemical detection inside monolithic capillary columns has been provided previously [19]. Briefly, a carbon fiber as working electrode and a silver microwire as a pseudo-reference electrode, with diameters of 7 μ m and 25 μ m, respectively, were attached to silver-plated wires using conductive silver paint. Afterwards, the microelectrodes were fixed in parallel on ceramic slides using cyanoacrylate adhesive. The capillary monolithic column was carefully slid to required length onto the working and reference electrode. The positions of the capillary and the embedded microelectrodes were fixed on a ceramic support with cyanoacrylate adhesive afterwards.

A similar protocol has been used to prepare a 3D-printed device with integrated electrochemical detection. Microelectrodes were fixed inside an empty fused-silica capillary (65 \times 0.32 mm) that was connected to the outlet of one of the channels of the titanium device (100 \times 0.8 mm) [19].

2.6. Sample preparation and extraction

The standard solutions of dopamine precursors and metabolites were prepared in mixtures of acetonitrile and water containing 0.1% TFA. The working samples were freshly prepared by appropriate dilution of the standard solution. Urine sample was collected from a healthy volunteer. Dopamine was extracted by using BondElut PBA and PSA cartridges (Agilent, Palo Alto, CA, USA) according to a provided protocol with one modification. Instead of diluting 1 ml of urine with 5 ml of deionized water, 5 ml of urine were used directly. Recovery rate of applied extraction cartridges for dopamine was $102.7 \pm 3.2\%$ ($n=3$).

2.7. Optimization of the mobile phase composition

To describe an effect of the composition of the mobile phase on the retention we have fitted retention data ($\log k$) determined in mobile phases containing 5, 20, 40, 60, 80, 90 and 95% of acetonitrile by a semi-empirical three-parameter model [28,33–36]:

$$\log k = a + m_{RP} \cdot \varphi_{H_2O} - m_{HILIC} \cdot \log \varphi_{H_2O} \quad (1)$$

where k is the retention factor of an individual dopamine-related compound and φ is the volume fraction of water in the mobile phase. Constant a correlates to the size of the molecule and to the analyte-stationary-mobile phase interaction energy, m_{RP} describes the interaction between solutes and solvents, and parameter m_{HILIC} is mainly related to analyte-stationary phase interactions. It has been shown that Eq. (1) provides a more accurate description of the retention than polynomial empirical equations [36].

Regression of the three-parameter retention model was performed in Origin 9.0 (OriginLab, Northampton, MA, USA). Regression analysis has been optimized using the probability value, p , at $\alpha = 0.95$, residual sum of squares, and adjusted correlation coefficient. If necessary, outliers were omitted in the model to improve the quality of regression. Generally, at most one experimental point with the highest residual value was removed. Table SI-1 (Supplementary Information) shows regression parameters together with quality-of-fit descriptors, which confirm successful fitting of the experimental data with the three-parameter model.

By using best-fit regression parameters of Eq. (1), the retention factor can be predicted for a given compound at any composition of the mobile phase, allowing the chromatographic resolution, $R_{1,2}$, of any two compounds to be calculated from Eq. (2). Here, n is the plate number of the column determined for the least-retained 2-phenylethylamin (2-PEA) in all mobile phases tested and extrapolated by a polynomial model across the entire range of mobile phases ($n=4\ 000$ to $5\ 000$), and k_1 and k_2 are retention factors of the former and latter peak, respectively.

$$R_{1,2} = \frac{\sqrt{n}}{4} \left(\frac{k_2 - k_1}{k_1} \right) \left(\frac{k_1}{1 + k_1} \right) \quad (2)$$

By plotting the calculated resolution of all pairs of peaks vs. the volume fraction of the acetonitrile in the mobile phase, one obtains window diagram summarizing an effect of the composition of the mobile phase on the resolution. From such a window diagram, we may establish composition ranges that show the highest possible resolution. The higher the window is the higher is the resolution of two respected peaks and the best separation is achieved in the highest point of any window. In the present study, a minimal resolution of $R_{1,2} = 1$ has been selected as an optimization criterion. Fig. 2 shows constructed window diagrams used for the determination of the optimal composition of the mobile phase on all four columns.

A Gaussian function has been used to predict peak shapes using calculated retention times (Eq. (1)) and peak widths (determined from plates number). The sum of the elution profiles for individual peaks yields the predicted separation at an optimal composition of the mobile phase [37].

3. Results and discussion

To develop an analytical device that allows parallel analyses of a single sample, we first optimized the composition of the mobile phase, so as to find the percentage of acetonitrile that provides the best resolution of all compounds on the four different monolithic stationary phases. Then, the flow of sample and mobile phase were split to simultaneously inject a single sample on all four monolithic capillary columns. After optimization of the experimental conditions, the four-channel 3D-printed titanium separation device, con-

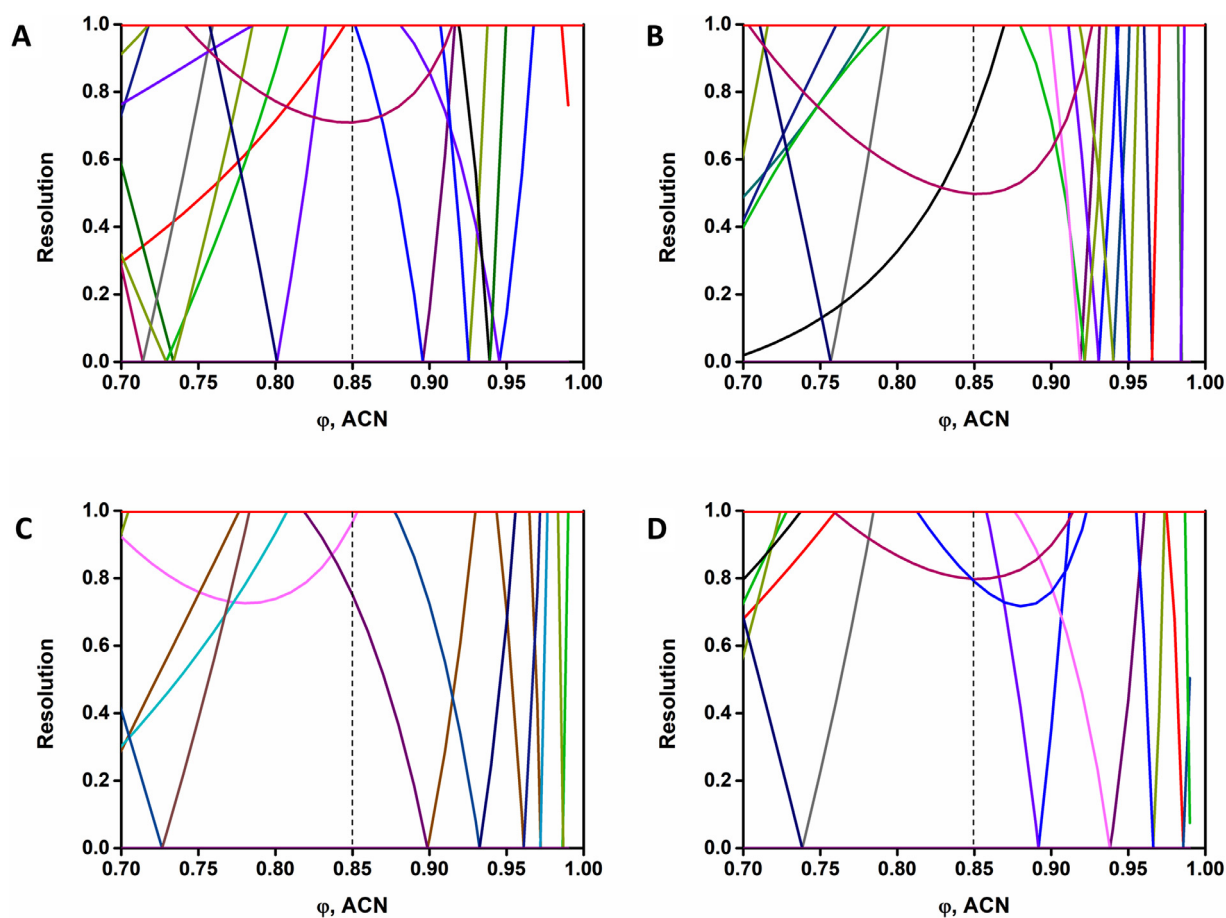


Fig. 2. Window diagram for monolithic capillary column prepared by using zwitterion sulfobetaine monomer and A) tetramethylene dimethacrylate, B) hexamethylene dimethacrylate, C) dioxyethylene dimethacrylate, and D) bisphenol-A glycerolate dimethacrylate crosslinking monomer. Resolution ($R_{1,2}$) – resolution of peak pair calculated using Eq. (2), ϕ (ACN) – concentration of the acetonitrile in the mobile phase. Black dashed line corresponds to selected mobile phase with 85% of acetonitrile. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

taining polymer monoliths and featuring integrated electrochemical detection, was applied.

3.1. Optimization of stationary-phase and mobile-phase composition

One of the main advantages of polymer-based monolithic stationary phases is the easy control of surface chemistry. Various protocols, including the selection of specific functional monomers, post-polymerization chemical modification, and/or surface-initiated grafting are generally applied [38]. On the other hand, each of these protocols require specific experimental conditions, such as reaction time and temperature, UV transparency of the capillary, or desired chemical stability and reactivity.

Since our final goal is the preparation of stationary phases in a four-channel titanium-based 3D-printed analytical device, we could not consider UV irradiation, and, hence, we had to discard grafting reactions as a surface modification method. Additionally, we preferred to select one polymerization reaction temperature and time, so as to minimize the number of experimental steps necessary for the fabrication of various stationary phases in a final device. Therefore, we chose an approach using different compositions of the polymerization mixture to prepare various monolithic stationary phases.

Monolithic stationary phases prepared by using a zwitterion sulfobetaine functional monomer have been found to provide a dual retention mechanism, combining both reversed-phase and hydrophilic-interaction effects, depending on the composition of

the mobile phase [28,33]. In mobile phases with a high concentration of water a reversed-phase retention mechanism dominates, while in acetonitrile-rich mobile phases retention is in line with hydrophilic-interaction liquid chromatography.

First, we prepared monolithic stationary phases using dioxyethylene dimethacrylate crosslinker [33,39] with several functional monomers, including lauryl methacrylate, glycidyl methacrylate, 2-methacryloyloxyethyl phosphorylcholine [40], and N,N-dimethyl-N-metacryloyloxyethyl-N-(3-sulfopropyl) ammonium betaine [33]. It proved difficult to prepare homogenous and permeable monolithic stationary phases using phosphorylcholine monomer, while stationary phases based on lauryl methacrylate or glycidyl methacrylate did not provide enough retention and selectivity for dopamine precursors and metabolites. Only columns prepared with zwitterionic sulfobetaine monomer provided the desired dual retention mechanism, combining both reversed-phase and hydrophilic-interaction effects [33,39,41]. Thus, we selected this monomer for further preparation of monolithic stationary phases.

Besides through the chemistry of the functional monomer, chromatographic properties of monolithic capillary columns can be also controlled by the type of crosslinking monomer, as demonstrated previously [33,39]. Therefore, we prepared monolithic stationary phases with zwitterion sulfobetaine functional monomer and tetramethylene dimethacrylate, hexamethylene dimethacrylate, dioxyethylene dimethacrylate, and bisphenol-A glycerolate dimethacrylate crosslinking monomers [33].

The effect of the mobile-phase composition on the retention and selectivity of dopamine precursors and metabolites was explored in order to find compromise isocratic selectivity for the dopamine molecules tested. By plotting the resolution calculated for individual peak pairs vs. the volume fraction of acetonitrile in the mobile phase, a window diagram summarizing the effect of the composition of the mobile phase on the resolution was obtained,

as shows Fig. 2. Here, we are looking at areas with no resolution lines below the desired resolution value ($R_{1,2} > 1.0$). An optimal mobile-phase composition providing separation with a chosen resolution is then selected within these areas. All columns provided better selectivity in acetonitrile-rich mobile phases with a HILIC retention mechanism. This is not surprising since all tested compounds are rather polar.

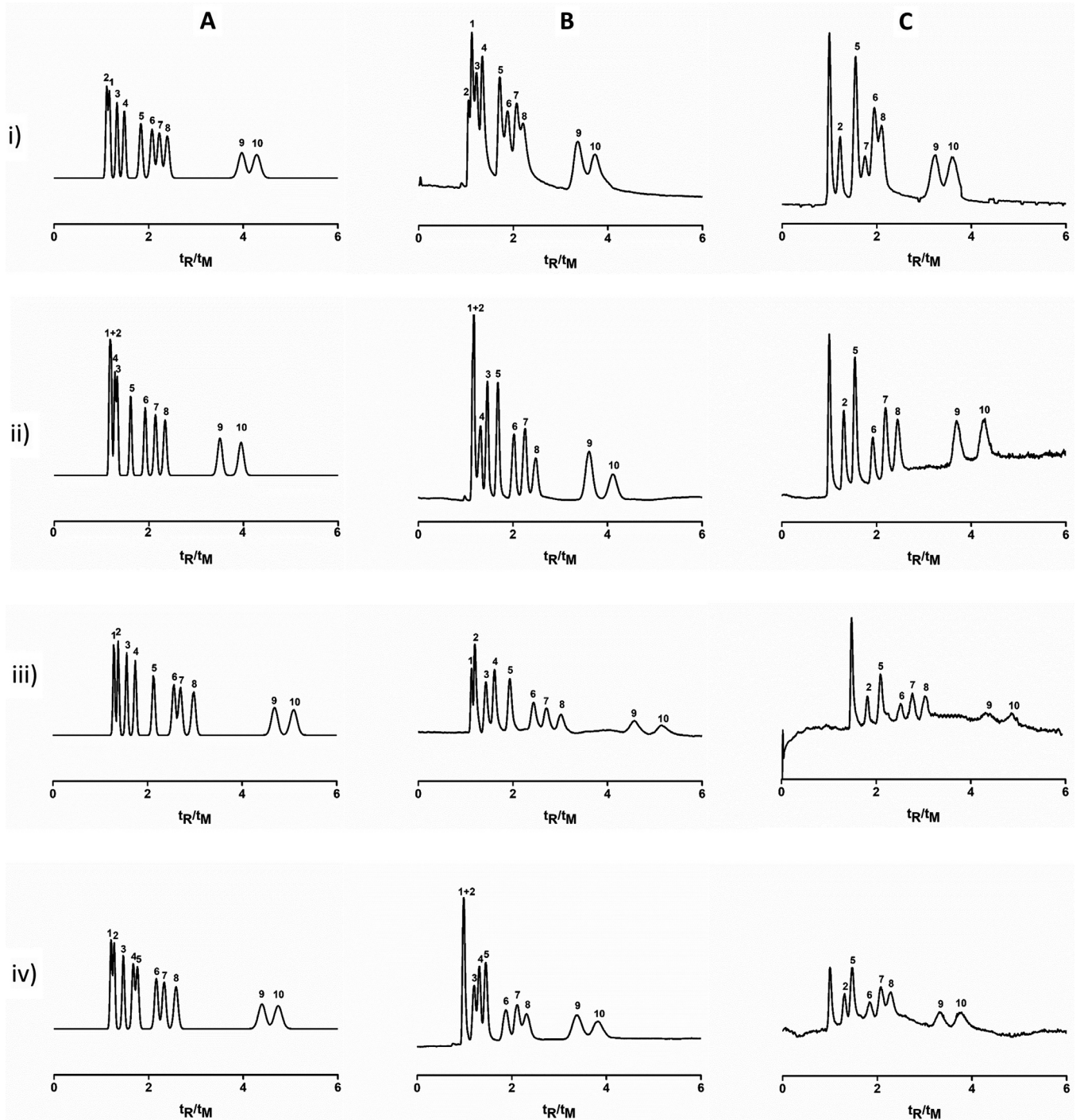


Fig. 3. Comparison of isocratic separations of dopamine precursors and metabolites predicted (A) by using best-fit parameters of Eq. (1), and measured in a mobile-phase-split experimental setup with UV (B) and electrochemical (C) detection. Monolithic phases were prepared by using zwitterion sulfobetaine monomer and i) tetramethylene dimethacrylate, ii) hexamethylene dimethacrylate, iii) dioxethylene dimethacrylate, and iv) bisphenol A glycerolate dimethacrylate crosslinking monomer. Mobile phase: 85% acetonitrile, flow-rate (before splitting) 25 μ L/min, back pressure 5.3 MPa. Analytes: 1 – 2-phenylethylamin hydrochloride, 2 – homovanillic acid, 3 – L-phenylalanine, 4 – tyramine, 5 – 3,4-dihydroxyphenyl acetic acid, 6 – tyrosine, 7 – dopamine hydrochloride, 8 – epinephrine hydrochloride, 9 – 3,4-dihydroxy-L-phenylalanine, 10 – norepinephrine hydrochloride.

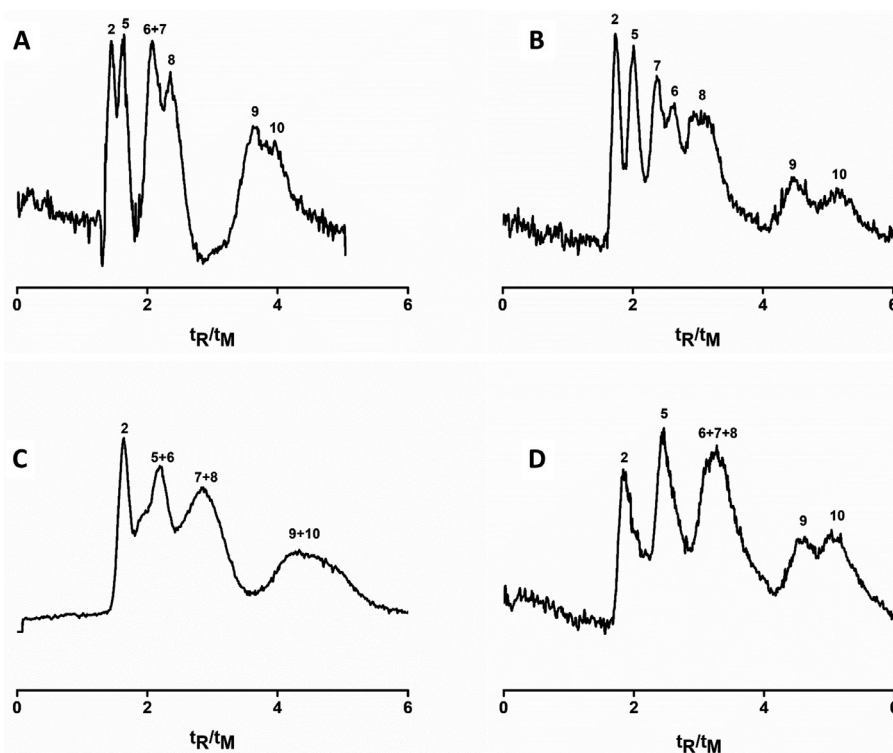


Fig. 4. Simultaneous separation of dopamine precursors and metabolites on monolithic stationary phases in four-channel 3D-printed titanium device with miniaturized electrochemical detection. Monolithic phases were prepared by using zwitterion sulfobetaine monomer and A) tetramethylene dimethacrylate, B) hexamethylene dimethacrylate, C) dioxyethylene dimethacrylate, and D) bisphenol-A glycerolate dimethacrylate crosslinking monomer. Mobile phase: 85% acetonitrile, flow-rate (before splitting) 25 $\mu\text{L}/\text{min}$, back pressure 3.4 MPa. Analytes: 1 – 2-phenylethylamin hydrochloride, 2 – homovanillic acid, 3 – L-phenylalanine, 4 – tyramine, 5 – 3,4-dihydroxyphenyl acetic acid, 6 – tyrosine, 7 – dopamine hydrochloride, 8 – epinephrine hydrochloride, 9 – 3,4-dihydroxy-L-phenylalanine, 10 – norepinephrine hydrochloride.

All columns showed the best resolution for mobile phases containing more than 95% acetonitrile. However, this composition of the mobile phase provided very high retention and peak broadening, which ruled it out for practical applications. Therefore, we selected a mobile phase with 85% acetonitrile as a compromise between resolution and speed of the analysis (retention) for the tested compounds. None of the columns could separate all molecules in a single run in the selected mobile phase and at least one pair of peaks was found to co-elute. The best resolution was provided by columns based on butanediol and hexanediol dimethacrylate crosslinking monomers, followed by diethylene glycol and bisphenol-A glycerolate monomers.

3.2. Parallel capillary liquid chromatography of a single sample

The goal of our study was the development of a separation device that allowed multiple simultaneous separations and associated detection of a single sample. Admittedly, commercially available multiport valves exist that allow injection of the same sample on more columns (see www.vici.com or www.thermofisher.com for more information). In the present experiments we tested the possibility to use a single-loop injection valve with a fixed volume of 200 nL, followed by two-stage flow splitting using T-pieces and small-internal-diameter connection capillaries. By using one T-piece at the first level and two at the second level (See Fig. 1) we were able to divide the sample across four capillary columns in a parallel arrangement. The total extra-column volume of the connection tubing between the injector and a device inlet following one flow path is circa 80 nL.

Separations predicted based on the best-fit parameters for Eq. (1) (Fig. 3A) were in good agreement with experimental data (Fig. 3B) obtained using a mobile-phase split arrangement and a

UV detector. It should be noted, however, that effects of extra-column volume, sample concentration, and actual column efficiency are neglected in the prediction since regression parameters for Eq. (1) were determined through direct injection of the standard solutions on capillary columns. Besides L-DOPA and norepinephrine, which always elute last, the other compounds are separated differently on the four stationary phases, once again confirming that the selectivity of monolithic stationary phases can be tuned by varying the composition of the polymerization mixture used for their preparation.

An electrochemical signal can easily be detected in a multichannel arrangement, while multichannel UV-Vis detectors are also available [42]. We have already demonstrated in previous work that electrochemical detection based on carbon-fiber and silver-microwire electrodes can easily be integrated in the monolithic capillary columns [19,20]. The separation traces shown in Fig. 3C for a single sample injection with electrochemical detection in a mobile-phase-split arrangement are simplified, because some of the compounds are not electroactive and thus do not yield a signal. This drawback can be solved in the future by combining integrated electrochemical detection with additional on-capillary detection methods [8,11]. This would also significantly enhance the analytical information provided by the system. The permeability of individual columns proved to be comparable [33]. However, due to the integration of miniaturized electrodes at the outlets of the columns disabling actual mobile phase flow-rate determination, we do not have precise control over the exact splitting ratio of the mobile phase. Therefore, the retention times should be normalized using the hold-up time of each column determined by a non-retained marker. To ensure repeatable splitting of the mobile phase and sample in the future, low internal diameter capillaries need to be inserted in between injection valve and column that will work

as mobile phase restrictors providing constant mobile phase flow-rate in all channels.

Fig. 3B and C confirm that monolithic capillary column with integrated electrochemical detection can be successfully applied for the parallel analysis of a single sample on four monolithic capillary columns, resulting in a compact instrument, combining both separation and detection in a single device.

3.3. Parallel analysis of a single sample on a 3D printed device

After the successful application of a mobile-phase split arrangement using monolithic capillary columns with both UV and electrochemical detection, we proceeded with the development of a titanium-based 3D-printed separation device. In the first generation of the titanium device, outlets of the channels were flat-ended, and we tested attachment of the microelectrodes perpendicular to the effluent stream. However, problems arose with the electrical isolation of the microelectrodes from the titanium backbone. Also, superglue was found not to offer a stable solution for fixing the microelectrodes to a metal surface when highly organic mobile phases were used.

The second generation of the device was fabricated with screw-threads at both inlet and outlet of the mobile phase flow (Fig. 1). Microelectrodes were again attached to a ceramic pad, inserted in empty fused-silica capillaries (65 × 0.32 mm), and attached to the outlets of the device using the threads, mimicking an integration of microelectrodes inside the monolithic capillary columns, as showed previously [19]. Such an experimental arrangement increased the extra-column volume of the system. The current manual method for fabricating the electrochemical detectors is limited to a certain minimal diameter of the detection capillaries (0.32 mm). In future work, we recommend instrumental micro-manipulation of the microelectrodes during detector preparation. Additionally, the efficiency of the prepared monolithic stationary phase is negatively affected by rough surfaces that selective laser melting produces and an internal diameter of the 3D-printed titanium channels (0.8 mm) as polymerization heat transfer is not effectively controlled in larger diameter scaffolds when compared to generally applied fused-silica capillaries [43,44]. Further improvements in metal printing, such as micro-selective laser melting [45] can enable the fabrication of channels with diameters down to 0.1 mm, thus offering more controlled heat transfer. The preparation of the monolithic stationary phase inside the titanium device may be also optimized [44,46] to improve the separation properties of the polymer monoliths.

Notwithstanding the current imperfections, Fig. 4 shows electrochemical traces of standard mixtures separated on monolithic stationary phases inside the microfluidic multi-channel titanium device, providing evidence of the successful development of parallel-single sample analysis on different monolithic stationary phases with miniaturized electrochemical detection. Homogeneity of the monolithic internal structure has to be further controlled in order to improve quality of separation achieved on presented separation device with an electrochemical detection (Fig. 3 in [19]).

As a final step in the current device development we explored the possibility of a quantitative analysis of dopamine in urine. In order to minimize a matrix effect, we applied a standard-addition method as shown in Table 1. Encouragingly, all four channels with integrated detectors provided comparable concentrations of dopamine in urine. Specifically, channels filled with monolithic stationary phases based on tetramethylene, hexamethylene, dioxyethylene, and bisphenol-A glycerolate dimethacrylate yielded dopamine values of 0.586, 0.594, 0.592, and 0.591 mg/L which is above previously determined limit of detection [19]. Run-to-run repeatability expressed as a relative standard deviation of peak heights (RSD, $n = 3$) is in the range of 0.03–1.71%.

Table 1

Run-to-run repeatability and determination of dopamine in human urine by a standard-addition method on the developed four-channel 3D-printed titanium device. Monolithic stationary phases were prepared by using zwitterion sulfobetaine monomer and A) tetramethylene dimethacrylate, B) hexamethylene dimethacrylate, C) dioxyethylene dimethacrylate, and D) bisphenol A glycerolate dimethacrylate crosslinking monomer. 0 – Urine sample extracted on BondElut PBA (Agilent, Palo Alto, CA, USA) cartridges, I – Extracted 5 mL of urine + 100 μ L of dopamine standard solution (0.5 mg/mL), II – Extracted 5 mL of urine + 200 μ L of dopamine standard solution (0.5 mg/mL), III – Extracted 5 mL of urine + 300 μ L of dopamine standard solution (0.5 mg/mL). I, nA – peak height, Mean, nA – arithmetic mean of measured peak heights, RSD, % – relative standard deviation.

	0	I	II	III
A				
I, nA	0.89	5.62	14.19	19.67
	0.88	5.73	14.26	19.78
	0.90	5.86	14.32	19.96
mean, nA	0.89	5.74	14.26	19.80
RSD, %	0.92	1.71	0.37	0.60
B				
I, nA	0.19	5.20	8.92	14.20
	0.20	5.17	8.94	14.19
	0.20	5.21	8.96	14.17
mean, nA	0.20	5.19	8.94	14.19
RSD, %	1.10	0.33	0.15	0.08
C				
I, nA	0.49	6.00	13.10	18.42
	0.50	5.98	13.00	18.43
	0.49	5.96	13.05	18.37
mean, nA	0.49	5.98	13.05	18.41
RSD, %	0.77	0.27	0.31	0.14
D				
I, nA	0.59	5.44	12.00	17.24
	0.59	5.43	12.00	17.22
	0.58	5.42	11.98	17.23
mean, nA	0.58	5.43	11.99	17.23
RSD, %	0.66	0.10	0.09	0.03

If applied to determine dopamine in clinical diagnostics, presented device might be used only after incorporation of proper extraction method. However, if we aim for a clinical diagnostics of dopamine-related compounds sensitive extraction method has to be incorporated first, together with improvement of sample separation quality, and minimization of extracolumn volumes.

4. Concluding remarks

In this work, we developed a compact multichannel separation device based on monolithic stationary phases and miniaturized electrochemical detection for parallel analyses of a single sample. We prepared four different polymer monoliths and we investigated the retention of ten dopamine precursors and metabolites. The composition of the mobile phase was rigorously optimized by creating a window diagram and a mobile phase containing 85% of acetonitrile was selected. The flow of sample and mobile phase was split using a two-stage arrangement of T-piece flow splitters, which allowed the simultaneous analysis of one sample on four different monolithic capillary columns. Both UV and electrochemical detection have been used and the resulting chromatograms were successfully compared with separations predicted using a three-parameter retention model.

Finally, we developed a four-channel 3D-printed titanium device to explore future possibilities for miniaturized analytical systems that combine monolithic stationary phases with multichannel electrochemical detection. A single sample of dopamine precursor

sors and metabolites was simultaneously separated on four independent stationary phases using the developed device. Dopamine levels in a human-urine sample were determined by multichannel analysis.

Further developments are necessary to (i) improve mobile-phase splitting by integrating flow splitting and distribution in a 3D-printed device, (ii) reduce the extra-column volume by micromanipulation of electrodes in narrower detection capillaries or their direct attachment to a separation part of the device, (iii) improve the efficiency of monolithic stationary phases prepared within the confinement of a titanium device, and (iv) composition of applied polymerization mixtures has to be tuned to prepared monolithic stationary phases allowing isocratic separation of tested compounds in both reversed-phase and hydrophilic interaction liquid chromatography. Nevertheless, the presented device constitutes a significant step towards the development of compact analytical systems for practical applications, such as dopamine-metabolism-related medical diagnosis and treatment monitoring.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.chroma.2019.460537](https://doi.org/10.1016/j.chroma.2019.460537).

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