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# Short Communication

# Effect of carbon in-line filters on catecholamine analysis by high-performance liquid chromatography with coulometric electrochemical detection

Michael Bubser*

*Zoological Institute, Neuropharmacology Division, University of Tübingen, Mohlstrasse 54/1, W-7400 Tübingen (Germany)*

Marieluise Bartmann

*Department of Pathophysiology of Vision and Neuro-Ophthalmology, Division of Experimental Ophthalmology, University of Tübingen, Ob dem Himmelreich 9, W-7400 Tübingen (Germany)*

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ABSTRACT

Biogenic amines were analysed by high-performance liquid chromatography with electrochemical detection according to the method of Kilpatrick *et al.* [*J. Neurochem.*, 46 (1986) 1865]. Equal amounts of structurally related catechols, *i.e.* noradrenaline (NA), dopamine (DA), dihydroxybenzylamine (DHBA) and dihydroxyphenylacetic acid (DOPAC), produced electrochemical signals of unequal sizes. Removal of the carbon in-line filter preceding the analytical electrochemical cell markedly increased the peak areas for NA, DA and DOPAC. For DA, this filter effect was large at pH 4.80 and gradually diminished at lower pH (4.60, 4.42, 4.20). These results indicate that the efficiency of electrochemical detection of catechols can be compromised by the use of carbon in-line filters.

## INTRODUCTION

High-performance liquid chromatography with electrochemical detection (HPLC–ED) is commonly used for the separation and subsequent analysis of biogenic amines derived from rat brain. Biogenic amines can be oxidized almost completely with a coulometric detector [1,2]. Thus, a coulometric detector should produce similar electrochemical responses when equal amounts of structurally related compounds, such as the catechols noradrenaline (NA), dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and dihydroxybenzylamine (DHBA), are analysed. When we used HPLC–ED according to Kilpatrick *et al.* [1], we found that this assumption is not valid for NA, DA and DOPAC. This paper describes these observations and tries to elucidate the factors contributing to this effect.

## EXPERIMENTAL

The HPLC–ED system consisted of a Bischof 2200 analytical pump, a pulse dampener, a Rheo-

* Corresponding author.

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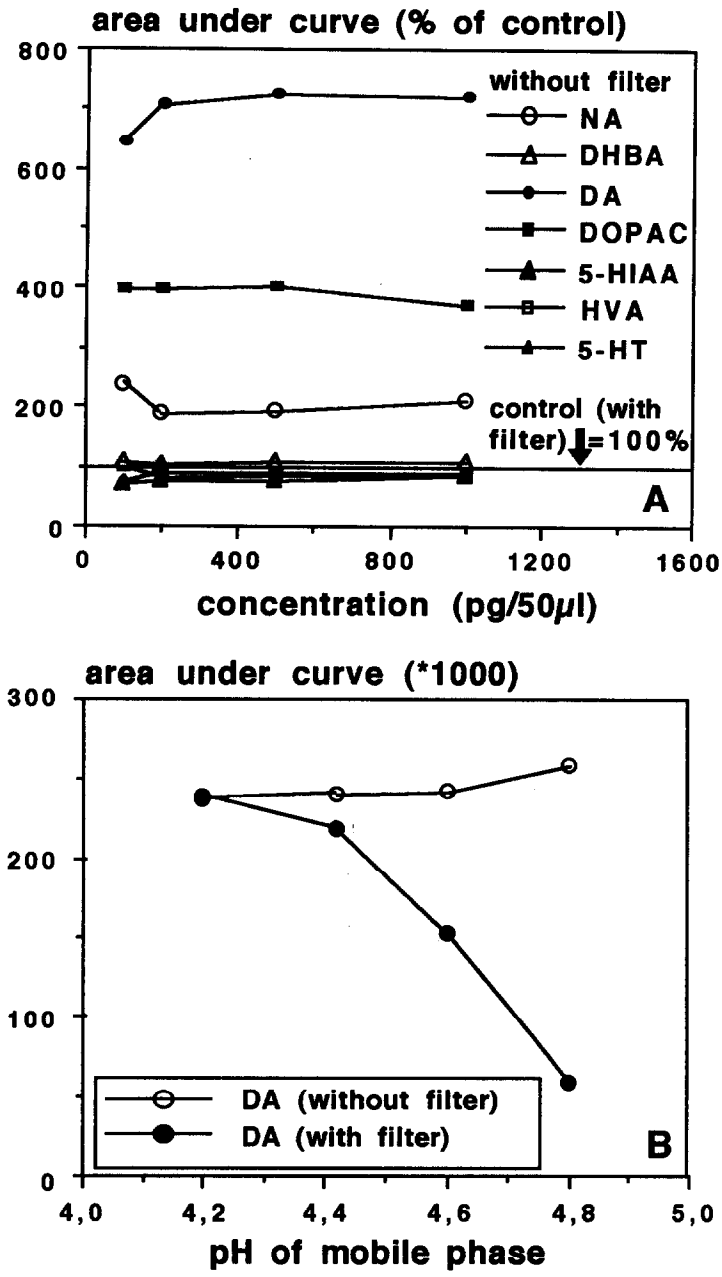


Fig. 1. (A) Effects of the carbon in-line filter on the detection of biogenic amines. A 50-µl volume of a solution containing 100, 200, 500 or 1000 pg of each standard was injected when the in-line filter was either absent or present. The results are presented as the percentage of each control (carbon in-line filter present). Data are means of two or three determinations run at pH 4.42. Standard errors of the mean were in all cases smaller than the respective symbols, and are therefore not presented. (B) pH-dependent effect of the carbon in-line filter on the electrochemical detection of dopamine. DA (1000 pg per 50 µl) was injected when the carbon in-line filter was either present or absent. Mobile phases were used in the following order: pH 4.80, 4.60, 4.42 and 4.20. There was an equilibration period of at least 30 min following mobile phase changes. Data are means of two determinations.

dyne 8125 injection valve with 100-μl sample loop and an ESA 5100A electrochemical detector (Bischof, Leonberg, Germany). An ESA 5020 guard cell ($E = +0.45$ V) was placed between the pulse dampener, and the injection valve. The ESA 5011 analytical cell was used in the screen mode (detector 1, $E = +0.02$ V; detector 2, $E = +0.32$ V). Carbon in-line filters were placed before the guard cell and the analytical cell [1,2]. Whenever chromatograms were run without the carbon in-line filter, this filter was replaced by an SSI adapter (Bischof). Nucleosil 5-$C_{18}$ (Bischof) was used as the stationary phase in both the analytical column (250 mm × 4.6 mm I.D.) and the guard column (20 mm × 4 mm I.D.). The mobile phase (flow-rate 1 ml/min) consisted of 6.973 g of sodium acetate, 7.355 g of citric acid monohydrate, 0.048 g of EDTA, 0.050 g of sodium octanesulphonic acid and 70 ml of methanol, made up to a final volume of 1 l with HPLC water [1]. The pH of the mobile phase was set at pH 4.42 with citric acid [3]. Axxiom 727 chromatography software (Sykam, Gilching, Germany) was used for data analysis.

In experiment 1, we tested whether the carbon in-line filter preceding the analytical cell affected the detection of the biogenic amines, DA, DHBA, DOPAC, homovanillic acid (HVA), NA, serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) under standard conditions, *i.e.* at pH 4.42 [3]. In experiment 2, we investigated whether the effects of the carbon in-line filter are dependent on the pH of the mobile phase, because most HPLC methods employing coulometric detection use more acidic mobile phases [4,5] and a high pH is known to facilitate the binding of catcholamines to glassy carbon [6]. Mobile phases with different pH (4.80, 4.60, 4.42 and 4.20) were prepared by adding either citric acid or sodium hydroxide to the mobile phase described above. Since changes of pH impair the chromatographic separation of compounds, the effect of pH on the detection of catecholamines was investigated for DA only.

## RESULTS AND DISCUSSION

In experiment 1, the NA, DOPAC and DA peaks, but not the DHBA, HVA, 5-HIAA and 5-HT peaks differed in height and area depending on whether the chromatograms were run with or without the carbon in-line filter in the HPLC–ED system. For all concentrations tested, the NA, DOPAC and DA peaks increased when the carbon in-line filter was removed (Fig. 1A) and were then in the range expected from theoretical considerations [2]. In experiment 2, changes of the pH of the mobile phase affected the peak area for DA only when the carbon in-line filter was present (Fig. 1B). The reduction of the DA peak caused by the in-line filter was most pronounced at pH 4.80. When the pH was reduced to 4.60 or 4.42 this effect was attenuated and it could no longer be detected at pH 4.20. Once the filter had been exposed to pH 4.20, changes of pH no longer affected the size of the DA peak.

Taken together, these findings demonstrate for the first time that some catecholamines interact with the carbon in-line filter. This interaction has been attributed to the chemical reaction of catecholamines with fine particles derived from filter production (G. P. Cellerino, ESA International, personal communication). However, flushing of the filter with the mobile phase of pH 4.42, which, according to the manufacturer, removes these particles, did not prevent the interaction. The effect of the filter occurs irrespective of the amount of compound injected and is linear as long as the amount of compounds to be detected is not near the detection limit. In the case of the analysis of minor amounts of catecholamines, the carbon in-line filter may be replaced by a fibreglass in-line filter (Knauer, Berlin, Germany).

In summary, the present investigation demonstrates that the carbon in-line filter commonly used with and strongly recommended for coulometric electrochemical detectors interacts with some catecholamines in a pH-dependent fashion, thereby reducing the efficiency of the detection system.

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