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Determination of Ascorbic Acid in Nanolitre Samples by Means of Capillary Batch Injection Analysis

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Capillary batch injection analysis (CBIA) is applied to the determination of ascorbic acid in nanolitre samples at neutral pH. It is demonstrated that pulsed amperometric detection is superior to constant-potential amperometric detection with respect to signal stability. The calibration plot is linear within the studied concentration range between 0.5 mM and 5 mM and the reproducibility is characterized by a relative standard deviation of 0.80% for 10 successive injections of 188 nL of 3.5 mM ascorbic acid. The attainable sample throughput is up to 500 per hour. © **1998 John Wiley & Sons, Ltd.**

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INTRODUCTION

Batch injection analysis (BIA) has been introduced by Wang and Taha, (1991) as an analytical technique that is based on the injection of microlitre sample volumes onto a nearby flat detector surface which is immersed in a large-volume blank solution. Several research groups have demonstrated the analytical utility of this method in fields such as trace metal analysis (Brett *et al.*, 1996), enzyme-based bioelectrochemical studies (Amine *et al.*, 1993) or potentiometric determinations (Wang and Chen, 1994).

Recent tendencies in chemical analysis have been focused on the miniaturisation of the apparatus in order to obtain analytical information with a minimum sample and reagent consumption in the shortest time possible. The new technique of capillary batch injection analysis (CBIA) presented in this paper enables the determination of substances in nanolitre samples with a high sampling frequency. The importance of CBIA for biologically oriented studies will be demonstrated using ascorbic acid as the target compound.

EXPERIMENTAL

Apparatus. A schematic illustration of the CBIA system is shown in Fig. 1. The small liquid samples are handled in a fused-silica capillary (75, 100 or 150 μm i.d.), connected to a microlitre syringe of 0.5, 5 or 10 μL (Hamilton, Switzerland). The piston of the syringe is driven by means of a microprocessor-controlled device, EDOS 5221 (Eppendorf, Germany). The dispensing system allows the injection of volumes in the range between 20 nL and 4.7 μL and eight dispensing speeds ranging from 270 nL/s to 758 nL/s are available (5 μL syringe). The electrode tip–capillary distance is adjusted by screwing the capillary assembly upward and downward. The capillary can easily be introduced into or removed from the cell via a precisely positioned glass sleeve. The voltammetric cell used for amperometric detection was made of Plexiglas® and Teflon® and incorporates all necessary electrodes including a platinum microdisk electrode (d_{Pt} = 25 μm) and an Ag/AgCl/3 M KCl reference/counter electrode. Convection of the cell electrolyte can be accomplished by an electromechanically driven rotating cylinder. All experiments were done with an Autolab potentiostat (Eco Chemie, Utrecht, The Netherlands) equipped with a low current amplifier (ECD) module.

Reagents. Ascorbic acid was obtained from Aldrich (Steinheim, Germany). Operating solutions of ascorbic acid were prepared immediately before use. All measurements concerning ascorbic acid were performed in a 0.6 mM phosphate buffer (pH = 6.79) containing 0.1 M potassium chloride. All reagents were of analytical grade and solutions were prepared using doubly-distilled water.

RESULTS AND DISCUSSION

The performance characteristics of the CBIA system depend on parameters such as capillary outlet–electrode distance, injection volume, flow rate, presence or absence of additional convection and capillary/electrode dimensions. Suitable experimental conditions were derived from CBIA measurements performed using potassium ferrocyanide/0.1 M sodium sulphate as model system and applying an amperometric detection mode (E = +600 mV vs Ag/AgCl/3 M KCl). These studies led to an optimum parameter setting which was used for ascorbic acid

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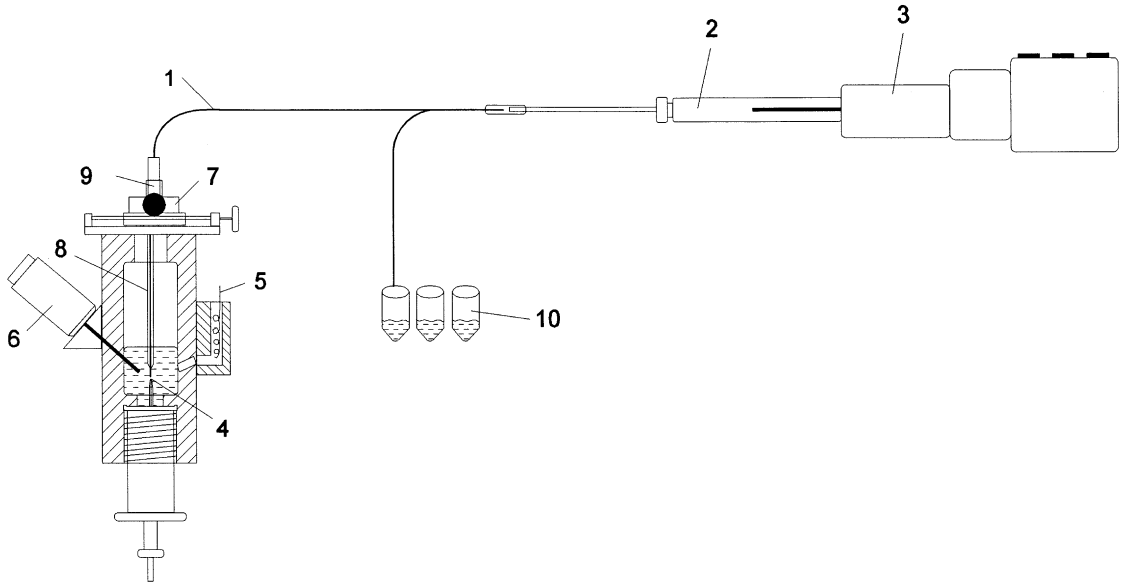

Figure 1. Schematic representation of the CBIA arrangement: (1) fused-silica capillary; (2) microlitre syringe; (3) EDOS 5221; (4) microdisk electrode; (5) reference/counter electrode; (6) motor connected with a rotating cylinder; (7) *x-y*-positioning device; (8) glass sleeve; (9) screw for *z*-positioning; and (10) sample vials.

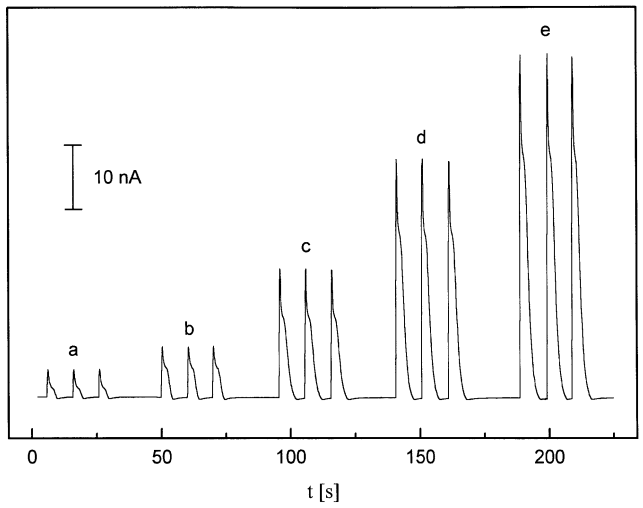

Figure 2. CBIA signals obtained for calibration of ascorbic acid: Ascorbic acid concentration [mol/l]: (a) 5.0×10^{-4}; (b) 1.0×10^{-3}; (c) 2.0×10^{-3}; (d) 3.5×10^{-3}; (e) 5.0×10^{-3}; injection volume, 188 nl; volume flow rate, 270 nl/s; capillary inner diameter, 100 μm; microelectrode tip–capillary distance, 200 μm; convection by means of stirring; pulse sequence for detection, $E_1 = +1200$ mV (50 ms), $E_2 = -500$ mV (50 ms), $E_3 = +900$ mV (100 ms), current sampling at E_3.

determinations as specified in Fig. 2. The neutral background buffer was chosen in order to adapt the measuring solution to physiological media.

In contrast to the ferrocyanide measurements amperometric detection at 0.6 V yield a progressive decrease of ascorbic acid signals for subsequent injections. Therefore, ascorbic acid determinations were performed applying multiple-pulse amperometric detection. An optimized pulse sequence was elaborated as given in Fig. 2 which ensures excellent baseline and signal stability. The precision of ascorbic acid determination is expressed by a relative standard deviation of 0.80% obtained for 10 repetitive injections of 188 nL of 3.5 mM ascorbic acid. The calibration plot corresponding to the CBIA recordings shown in Fig. 2 is linear characterized by a slope of 12.6 ± 0.3 nA/mM, an intercept of -3.4 ± 0.9 nA and a regression coefficient of 0.999 ($n = 5$). The maximum attainable sample throughput is as high as about 500 per hour.

In conclusion CBIA coupled with pulsed amperometric detection has been proved to be a reliable method for determining ascorbic acid in nanolitre samples at physiological pH. In comparison with other capillary-based methods such as capillary flow injection analysis (Liu and Dasgupta, 1992; Matysik and Werner, 1993) and capillary electrophoresis ascorbic acid determination by means of CBIA has the advantage to be very fast which is important to prevent partial oxidation of the analyte during the analytical process.

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