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Electrochemical droplet-based microfluidics using chip-based carbon paste electrodes for high-throughput analysis in pharmaceutical applications

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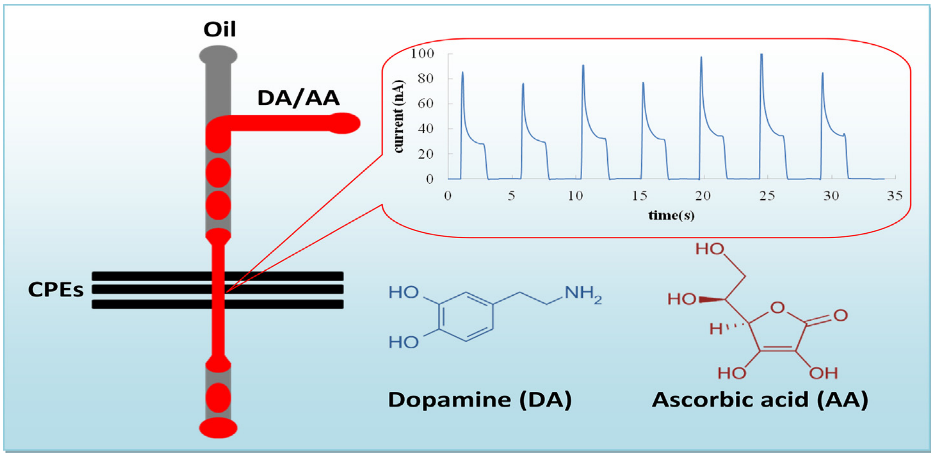
HIGHLIGHTS

- Droplet microfluidics with CPEs was developed for pharmaceutical applications.
- This system was used to quantitative analysis of DA and AA in intravenous drugs.
- Highly accurate and precise analysis of DA and AA using this system was achieved.

GRAPHICAL ABSTRACT



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ABSTRACT

This paper presents the first example of a pharmaceutical application of droplet-based microfluidics coupled with chronoamperometric detection using chip-based carbon paste electrodes (CPEs) for determination of dopamine (DA) and ascorbic acid (AA). Droplets were generated using an oil flow rate of 1.80 μL min^{-1}, whereas a flow rate of 0.80 μL min^{-1} was applied to the aqueous phase, which resulted in a water fraction of 0.31. The optimum applied potential for chronoamperometric measurements in droplets was found to be 150 mV. Highly reproducible analysis of DA and AA was achieved with relative standard deviations of less than 5% for both intra-day and inter-day measurements. The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 20 and 70 μM for DA and 41 and 137 μM for AA, respectively. Linearity of this method was in the ranges of 0.02–3.0 mM for DA and 0.04–3.0 mM for AA. This system was successfully applied to determine the amounts of DA and AA in intravenous drugs.

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Chronoamperometry
Electrochemical detection

Calibration curves of DA and AA for quantitative analysis were obtained with good linearity with R^2 values of 0.9984 and 0.9988, respectively. Compared with the labeled amounts, the measured concentrations of DA and AA obtained from this system were insignificantly different, with error percentages of less than ±3.0%, indicating a high accuracy of the developed method.

1. Introduction

Droplet-based microfluidic systems were introduced into scientific research a decade ago in order to overcome the classical problems of slow mixing and sample zone dispersion associated with laminar flow microfluidic platforms [1]. Since then the droplet technology has grown rapidly and been widely exploited in a diverse range of applications [2–5], such as chemical synthesis [6], biological and cell studies [7–10], medical applications and pharmaceutical sciences [11,12]. This is due to the fact that droplet-based microfluidic systems have successfully exploited the outstanding advantages of miniaturization, including small sample consumption, high analytical performance, low cost, portability, the potential for parallel analysis and short analysis times. In addition, droplet systems possess uniquely superb performance. For example, precisely defined volume droplets can be controllably produced at high generation frequencies up to 1 kHz or above. This renders droplet microfluidics a promising platform for high-throughput analysis, in which each isolated droplet can serve as a micro/nano reactor. Significantly, the compartmentalization of droplets not only prevents diffusion of sample zone, surface adsorption and cross-contamination [1,13], but also enhances rapid mixing [3,14]. These allow reactions occurring within droplets to be accurately and precisely controlled and monitored.

Apart from droplet generation and manipulation, the online detection and characterization of an individual droplet is also a challenge. There have been various detection methods, such as fluorescence spectroscopy [1,8,15], mass spectrometry [16,17], electrochemical detection [18–22], Raman spectroscopy [23–25], that have been used to characterize droplet contents. Among these detection techniques, fluorescence has been the most attractive detection system due to its high sensitivity and fast response. However, this detection method encounters the problems of complexity, high cost and large instrumentation which is not ideally compatible with microfluidic systems. Unlike other detection systems, electrochemical detection offers an alternative relatively simple, cheap, sensitive, selective and label-free method for droplet detection. Importantly, the size of the electrochemical detection setup is perfectly suitable to be incorporated with microfluidic systems. Accordingly, electrochemical detection holds a great promise to be a detection method for droplet-based microfluidics. To date, there have been limited published reports on electrochemical detection for droplet-based microfluidic systems. For example, Liu et al. [26] successfully demonstrated a chronoamperometric method using gold microband electrodes, to determine droplet contents and characterize droplet generation frequency, size and velocity. In addition, Han et al. [27] developed a droplet-based microfluidic system coupled with amperometric detection using Pt wire electrodes to monitor the kinetics of the decomposition of H_2O_2 by catalase occurring within droplets. Using this system, the complete Michaelis–Menten kinetics of catalase was successfully measured. Furthermore, Filla et al. [28] presented a corona discharge electrode to create a hydrophilic/hydrophobic interface in order to desegment the flow into separate oil and aqueous streams. The desegmented droplets' contents were subsequently analyzed using electrochemistry or microchip electrophoresis with electrochemical detection. Additionally, potentiometric [29] and coulometric [30] detection systems have also successfully been used for the analysis of droplet contents. Recently, highly reproducible chronoamperometric analysis of microdroplets containing 0.1 mM $Ru(NH_3)_6^{3+}$ was presented by Liu et al. [22]. A narrow section of the microchannel, which was placed across gold microband electrodes, was used to elongate droplets to attain reproducible current measurements.

In all situations, electrochemical detection methods have shown their abilities for analyzing droplet contents. However, all published reports have focused on metal electrodes. The main problems of using metal electrodes are fouling and narrow working potentials. Therefore, in this work, it was successfully demonstrated for the first time that chip-based carbon paste electrodes (CPEs) fabricated as three microbands within the microfluidic device can be used to monitor electrochemical activities within droplets. The chip-based CPEs were originally developed by Henry's research group [31]. Unlike metal electrodes, the CPEs have attracted considerable attention because of their unique property to withstand fouling and flow-based systems and they possess a larger potential range [31,32]. A confined channel design previously presented by Liu et al. [22] was also exploited to obtain reproducible chronoamperometric measurements within droplets. Using this approach, chronoamperometric measurements of dopamine and ascorbic acid in real samples of intravenous drugs were successfully achieved.

2. Experimental

2.1. Materials and chemicals

All chemicals were analytical grade. The following materials and chemicals were used as received: sodium chloride (NaCl: Merck, Thailand), disodium hydrogen phosphate (Na_2HPO_4: Merck, Thailand), potassium dihydrogen phosphate (KH_2PO_4: Carlo ERBA, Thailand), potassium chloride (KCl: Ajax Finechem, Thailand), Noujol mineral oil (PerkinElmer, Thailand), silver paint (SPI supplies, USA), graphite powder (≤20 μm, Sigma–Aldrich, Singapore), ethanol (Merck, Thailand) and poly(dimethylsiloxane) (PDMS) Sylgard 184 eleastomer kit (Dow Corning, USA). Potassium ferricyanide ($K_3[Fe(CN)_6]$) and potassium ferrocyanide ($K_4[Fe(CN)_6]$) were obtained from Sigma–Aldrich (Singapore). Dopamine hydrochloride (DA) and ascorbic acid (AA) were purchased from Sigma–Aldrich (Singapore) and BDH (AnalaR, England), respectively.

Intravenous drugs containing either DA or AA were purchased from the King Chulalongkorn Memorial Hospital (Thailand) and a local pharmacy. Three samples of DA which are Upamine (Umeda Co., Ltd., Thailand), Domine-250 (Modern Menu Co., Ltd., Thailand) and Dopamex (Biolab Co., Ltd., Thailand) were determined. The labeled amounts of DA contained in Upamine, Domine-250 and Dopamex are 250 mg/10 mL, 250 mg/10 mL and 200 mg/10 mL, respectively. A sample of ascorbic acid is a product of Atlantic Laboratories Corporation Ltd. (Thailand) and contains AA at a concentration of 500 mg/2 mL.

The oil solution for droplet generation was a 10:2 (v/v) mixture of perfluorodecalin (mixture of *cis* and *trans*, 95%, Sigma–Aldrich, Germany) and 1H, 1H, 2H, 2H-perfluoro-1-octanol (97%, Sigma–Aldrich, Germany). All aqueous solutions were prepared with deionized water (18.0 MΩ cm, Milli-Q Gradient System, Millipore, Thailand).