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Graphene-polyaniline modified electrochemical droplet-based microfluidic sensor for high-throughput determination of 4-aminophenol

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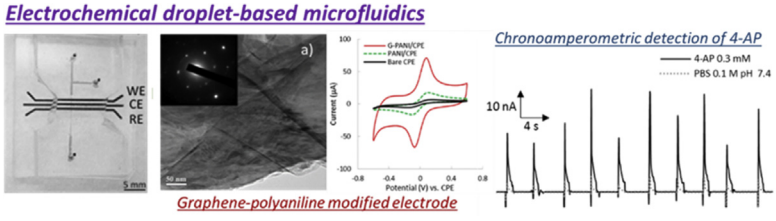
HIGHLIGHTS

- A novel combination of G-PANI/CPE and droplet-based microfluidic device for determination of 4-AP was achieved.
- G-PANI nanocomposite increases the surface area and improved electrochemical sensitivity of the electrode.
- This novel droplet-based system was successfully applied for 4-AP monitoring in commercial PA samples.

GRAPHICAL ABSTRACT



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ABSTRACT

We report herein the first development of graphene-polyaniline modified carbon paste electrode (G-PANI/CPE) coupled with droplet-based microfluidic sensor for high-throughput detection of 4-aminophenol (4-AP) in pharmaceutical paracetamol (PA) formulations. A simple T-junction microfluidic platform using an oil flow rate of 1.8 μL/min and an aqueous flow rate of 0.8 μL/min was used to produce aqueous testing microdroplets continuously. The microchannel was designed to extend the aqueous droplet to cover all 3 electrodes, allowing for electrochemical measurements in a single droplet. Parameters including flow rate, water fraction, and applied detection potential (E_{det}) were investigated to obtain optimal conditions. Using G-PANI/CPE significantly increased the current response for both cyclic voltammetric detections of ferri/ferrocyanide $[Fe(CN)_6]^{3-/4-}$ (10 times) and 4-AP (2 times), compared to an unmodified electrode. Using the optimized conditions in the droplet system, 4-AP in the presence of PA was selectively determined. The linear range of 4-AP was 50–500 μM ($R^2 = 0.99$), limit of detection (LOD, S/N = 3) was 15.68 μM, and limit of quantification (LOQ, S/N = 10) was 52.28 μM. Finally, the

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4-Aminophenol
Paracetamol

system was used to determine 4-AP spiked in commercial PA liquid samples and the amounts of 4-AP were found in good agreement with those obtained from the conventional capillary zone electrophoresis/UV–Visible spectrophotometry (CZE/UV–Vis). The proposed microfluidic device could be employed for a high-throughput screening (at least 60 samples h^{-1}) of pharmaceutical purity requiring low sample and reagent consumption.

1. Introduction

Since the mid-1940s, paracetamol (PA, acetaminophen or *N*-acetyl-*p*-aminophenol) has been commonly used as an antipyretic and analgesic drug around the world [1,2]. Many PA formulations, such as tablets, syrups, injections, and suppositories, have been available and highly effective for a variety of patients including children, pregnant women, and the elderly. Unfortunately, 4-aminophenol (4-AP) residue might be present as an impurity in PA formulations obtained from its synthesis or as a result of degradation [3]. Consuming 4-AP unintentionally can cause numerous pathologies (e.g. nephrotoxicity and hepatotoxicity) [3–5]. The European, United States, British, German, and Chinese Pharmacopoeias have set the maximum allowable limit of 4-AP in the PA drug formulations at 50 ppm (0.005% w/w) [5]. Several analytical approaches to determine 4-AP in PA formulations have been developed including high performance liquid chromatography (HPLC) coupled with UV–Visible [6,7] or fluorescence [8] detection, electrochemical detection [9], capillary electrophoresis [10,11], and flow-based analytical techniques [4,12]. Although these techniques offer highly selective, and sensitive quantification of 4-AP in PA formulations, these techniques are time consuming, have high operation costs, and are available only in sophisticated laboratories.

Recently, droplet microfluidic systems have held great promise for analytical measurements due to their portability, fast analysis time, low manufacturing cost, low reagent/sample consumption, and high-throughput screening [13]. Droplet microfluidic systems are capable of generating and manipulating small droplets encapsulated by an immiscible phase. Among other microfluidic platforms, droplet microfluidics has gained attention because of superior mixing efficiency and low dispersion/adsorption of sample within the microchannel [14]. The utility of droplet-based technology has been demonstrated for many applications, such as biomedical diagnosis [15,16], food safety [17,18], and environmental monitoring [19]. Other demonstrated applications include protein crystallization [20] and enzymatic kinetic assays [21], emulsion-based polymerase chain reaction [22], chemical synthesis [23,24], and single cell-based analysis [25]. For detection in droplet microfluidics, various detection methods have been used including laser-induced fluorescence [26], mass spectrometry [27], Raman spectroscopy [28], absorption spectroscopy [29], and bright-field microscopy [30]. Although these techniques offer highly selective, sensitive and accurate quantification, the main disadvantage are high equipment cost and challenge of coupling them with miniaturized systems for on-site applications.

Electrochemical detection is an attractive and alternative detection method for microfluidics because it is high speed, high sensitivity, cost-effectiveness, and independence of sample turbidity or optical path length [31]. Currently, there have been a few reports on electrochemical detection for droplet microfluidic systems. For example, Liu et al. used chronoamperometry for the detection of droplet contents and characterization of droplet generation (e.g. frequency, size and velocity) [32]. Han et al. developed an amperometric droplet microfluidic device to investigate the kinetics of enzymatic decomposition of H_2O_2 using catalase [33]. Gu et al. used a Pt-black modified electrochemical droplet-based microfluidic sensor for enzymatic glucose assays in biological fluids [34]. Itoh et al. demonstrated a novel droplet-based microdevice with an electrochemical ATP-sensing function for fish freshness determination [35].

The use of nanomaterials to improve electrode performance in microfluidic devices has drawn increasing attention in recent years. Numerous nanomaterials (e.g. gold nanoparticles, silver nanoparticles, single-wall carbon nanotubes, and multi-wall carbon nanotubes) are currently available and can increase active surface area of the working electrode, leading to enhance electrochemical sensitivity and conductivity [34,36–38]. Graphene (G) has received substantial attention for chemical modification of electrodes [39], owing to its large surface area, extraordinary electrical conductivity, high mechanical strength and potentially low manufacturing cost. In addition, G has been also used to modify electrode surface in order to improve the electrochemical properties of the electrodes. One problem associated with G-based electrode modification is the potential for self-agglomeration of pure G on the electrode surface. One solution is use of a nanocomposite consisting of conducting polymer and G to increase the distribution of graphene [40]. Among conducting polymers, polyaniline (PANI) has been widely used for electrode modification because of its inherent electrochemical properties, biocompatibility and stability [41,42]. Previous reports have used G-PANI hybrid modified electrochemical sensors for electrochemiluminescent detection of luminol [43], voltammetric determination of dopamine [41] and dobutamine [44], and electrochemical immunoassays for estradiol [45] and salbutamol [46]. To the best of our knowledge, there are no previous reports combining a novel G-PANI modified electrodes and droplet microfluidics for electrochemical determination of 4-AP.

Herein, the aim of this work is to develop a high-throughput and sensitive method using G-PANI modified electrochemical droplet-based microfluidic sensor for determination of 4-AP in pharmaceutical PA products. An improved selectivity for 4-AP was achieved using G-PANI modified carbon-paste electrodes (G-PANI/CPE) operating at the optimized detection potential for highly selective detection of 4-AP in the presence of PA. This system offers an alternative method to measure 4-AP level in acceptable concentration range. Finally, this approach was successfully applied for the determination of 4-AP in commercial PA liquid samples, giving high correlation with a CZE/UV–Vis method. Accordingly, measurements using this approach can provide a fast and high-throughput route and could be an ideal platform for screening of purity in pharmaceutical products with small sample consumption. Furthermore, the development of this microfluidic model can be further extended to various applications such as medical testing, environmental monitoring and quality control of foods or beverages.