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Electrochemical sensing of mesalazine and its *N*-acetylated metabolite in biological samples using functionalized carbon nanotubes

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ABSTRACT

A rapid analytical method without the time-consuming separation step was developed to simultaneously determine mesalazine and its *N*-acetylated metabolite. A simply designed electrochemical sensor with functionalized carbon nanotubes in a Nafion matrix was constructed for this purpose. The presence of the nanocomposite modifier on the electrode surface significantly affects the voltammetric response of target analytes. The morphology of the modified surface was investigated by scanning electron microscopy. The effect of modifier amount on the sensor performance was investigated in order to obtain the most favorable response of mesalazine since it was found in lower concentration limits in real samples then its metabolite due to the rapid drug elimination and the slightly slower renal metabolite excretion. Under optimal conditions, the anodic peak currents measured by square-wave voltammetry increased linearly after short accumulation of 30 s in the range of 5.0×10^{-8}–2.5×10^{-6} M and 1.0×10^{-7} – 5.0×10^{-6} M for drug and metabolite, respectively. In addition to stable response, the sensor has excellent performance associated with high sensitivity (2.33×10^{7} and 8.37×10^{6} μA M^{-1} for drug and metabolite, respectively). The synergistic effect of the carbon nanotubes and Nafion polymer film yielded detection limit of 1.2×10^{-8} M for mesalazine and 2.6×10^{-8} M for its metabolite that is comparable to known chromatographic methods. Due to the easy preparation and regeneration, the proposed sensor opens new opportunity for fast, simple and sensitive analysis of drug and its metabolite in human serum samples as well as direct quantification of mesalazine in delayed-release formulations.

1. Introduction

Mesalazine (5-aminosalicylic acid, 5-ASA) is an anti-inflammatory drug widely prescribed for the therapy of ulcerative colitis and Crohn's disease (Scheme 1). It may also provide protection against the development of colorectal cancer in patients suffering from inflammatory bowel diseases [1]. In addition, 5-ASA inhibits cell injury in the inflamed mucosa by scavenging reactive oxygen metabolites, thus suppressing their toxicity. The drug is absorbed quickly from the small intestine when administrated orally and therefore, modified-release dosage forms are designed to deliver drug in the terminal ileum and colon [2]. After absorption, 5-ASA is metabolized by *N*-acetyltransferase to its *N*-acetyled-5-ASA derivative (Ac-5-ASA) in the liver and intestinal mucosa. This compound is the major metabolite present in blood with a half-life of up to 10 h. In plasma, both 5-ASA and Ac-5-ASA are found 40–50% and 80%, respectively bound to proteins.

Various analytical methods were described in the literature for the determination of 5-ASA, such as liquid chromatography coupled to mass spectrometry for drug quantification in plasma samples [3], HPLC with UV detection for stability-indicating assays [4] and analysis in pharmaceutical formulations [5], capillary electrophoresis for drug impurity profiling [6], spectrophotometry [7], fluorescence spectroscopy [8], automated chemiluminescence [9] and ultra-performance liquid chromatography [10]. At present, several HPLC methods with a fluorescence [11–13], electrochemical [14,15] and mass spectrometry detection [16,17] were developed for simultaneous quantification of 5-ASA and its *N*-acetylated metabolite. The satisfactory results were obtained with excellent selectivity and low detection limit. However, the developed chromatographic methods require derivatization of drug and metabolite before detection step, tedious sample preparations, consumption of large solvent volumes and time-consuming procedures. In developed HPLC methods the analytical run time per sample took about 10–40 min.

The electroanalytical methods have proved to be useful for sensitive and selective determination of many pharmaceuticals owing to fast response, low instrument and analysis cost as well as simplicity of samples preparation [18,19]. In spite of that, no attempt has been made to this date to determine 5-ASA and its metabolite by an electroanalytical method. There are only a few

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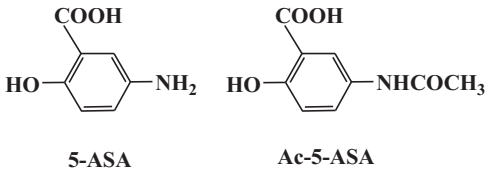

Scheme 1. Chemical structures of mesalazine (5-ASA) and its *N*-acetylated metabolite (Ac-5-ASA).

reports available on the electrochemical determination of 5-ASA. The sonolinear sweep voltammetric method was developed for determination of drug in tissue culture medium [20]. 5-ASA was quantified using glassy carbon electrode (GCE) [21] and pencil graphite electrode [22], however both methods were applied only to analysis of pharmaceutical formulations. The abrasive stripping voltammetry was used for identification of 5-ASA in commercial dosage forms [23], but proposed method was not quantitative. The amperometric catalase-peroxidase based biosensor was developed for measurement of 5-ASA concentration by indirect method monitoring dissolved oxygen level [24]. The high cost of enzymes used in the kit limits widespread application of the method for routine purpose. The oxidative behavior of 5-ASA was studied on the surface of GCE modified with nanoporous film and subsequently, electrochemically deposited polypyrrole using 1,5-naphtalenedisulfonic acid as dopant [25], however the developed method was designed only for quantitation of 5-ASA. Due to prolonged therapy and maintenance of remission in inflammatory bowel diseases, as well as the need for clinical and bioequivalence studies after the administration of newly developed delayed-release formulations, a simple, inexpensive and highly sensitive electroanalytical method is in great demand for simultaneous determination of 5-ASA and its metabolite.

Carbon based electrodes are commonly used in electroanalytical chemistry because of their low cost and wide availability. On the other hand, carbon nanotubes (CNTs) have attracted scientific interests in recent years owing to unusual mechanical strength and electrical conductivity, unique structure with huge surface area and excellent electrocatalytic activity [26]. Theirs exceptional properties offered versatile platform to employ them as the active material for the preparation of various composite electrode materials. CNTs-based sensors generally have higher sensitivities at low concentration levels or in the complex matrix, lower detection limits and faster electron transfer kinetics than traditional carbon electrodes. However, many factors need to be investigated in order to create an optimal CNTs-based sensor.

In the present study, the composite material based on the coupling of multi-walled CNTs and Nafion as cation exchange polymer was used to develop a fast and simple method for simultaneous measurement of 5-ASA and its metabolite without a separation step. The developed electrochemical sensor was successfully applied for simultaneous determination of 5-ASA and Ac-5-ASA metabolite at trace levels in serum samples using adsorptive stripping square-wave voltammetry (SWV). In addition, the proposed sensor was used to develop inexpensive and rapid method for direct measurement of active ingredient in different 5-ASA delayed-release formulations.

2. Experimental

2.1. Apparatus

Voltammetric measurements were performed using a μ-Autolab potentiostat (Eco Chemie, Utrecht, The Netherlands) controlled by GPES 4.9 software. A three-electrode cell system was used. The multi-walled CNTs and Nafion polymer modified GCE (CNTs-N/GCE) and a bare GCE (3-mm diameter, Metrohm, Switzerland) were used as a working electrode, a platinum wire and an Ag/AgCl/3 M KCl (Metrohm) were used as the counter and reference electrodes, respectively. In preliminary measurements, a bare gold electrode (2-mm diameter, Metrohm, Switzerland) and gold electrode modified in the same way as GCE were also employed as the working electrode. Prior to modification, the working electrode was polished with aqueous slurry of 0.05 μm alumina powder on a smooth polishing cloth, thoroughly rinsed with water and then ultrasonically cleaned in water for 30 s. Finally, the electrode was washed with purified water and dried. All electrochemical experiments were carried out at room temperature (23 ± 1 °C). When required, stirring was applied using a computer-controlled stirrer at ca. 300 rpm.

Scanning electron microscopy (SEM) measurement was peformed on a Jeol JSM-7000F microscope (Jeol Ltd., Tokyo, Japan).

2.2. Chemicals

5-ASA supplied by Merck (Darmstadt, Germany) was used without further purification. Ac-5-ASA metabolite, the multi-walled CNTs ($>98\%$, O.D. 6–13 nm, length 2.5–20 μm) and Nafion (5 wt% solution in a mixture of lower aliphatic alcohols and water) were obtained from Sigma-Aldrich (Steinheim, Germany). All other chemicals were of analytical grade quality. Ultra pure water used for the preparation of standard solutions and buffers was obtained by a Milli-Q system (Millipore, Bradford, USA). Salofak® (Dr. Falk Pharma, Germany) and Pentase® (Ferring GmbH, Germany) delay-released tablets containing 500 mg of 5-ASA were supplied from local pharmacy.

Stock solution of 5-ASA (1×10^{-3} M) was prepared by dissolving appropriate amount of the compound in purified water with addition of a drop of 1 M HCl to minimize the risk of 5-ASA oxidation [21] and stored in the dark under refrigeration. Stock solution of Ac-5-ASA (1×10^{-3} M) was prepared by dissolving its adequate amount in methanol. Standard solutions were prepared daily by diluting the stock solutions with a supporting electrolyte just before use. Britton–Robinson (BR) buffer solutions (0.04 M in each of acetic, phosphoric and boric acids) adjusted to the desired pH with addition of a 0.2 M NaOH were used as supporting electrolytes.

2.3. Preparation of CNTs-N/GCE

To generate carboxylic acid-functionalized surface the multi-walled CNTs (50 mg) were added to plentiful concentrated nitric acid and then sonicated for about 4 h. The suspension was filtered and washed with redistilled water to neutral. The solid powders were dried under vacuum at room temperature. One milligram of acid-treated CNTs was dispersed in 0.3% Nafion ethanol solution to give a 1.0 mg mL^{-1} black suspension with the aid of ultrasonic agitation. The nanocomposite film modified electrode was fabricated by dropping 3 μL of black suspension on cleaned working electrode surface and evaporating the solvent at room temperature. To obtain a stable cyclic voltammogram as well as strength CNTs adhesion to the electrode surface, the modified electrode was scanned prior to first measurement by two successive cyclic voltammetric sweeps between 0 and 1.5 V at 100 mV s^{-1} in a blank solution of BR buffer pH 2.0. The surface area of modified electrode was obtained by cyclic voltammetry (CV) using 1.0×10^{-3} M $K_3Fe(CN)_6$ in 0.1 M KCl electrolyte at different scan rates (ν) between -0.2 and 0.7 V. From the slope of the anodic peak current versus $\nu^{1/2}$ relation, the surface area of the CNTs-N/GCE was calculated to be 0.156 cm^2, which was about three times greater than the surface of the bare GCE. For comparison, the carbon nanotubes modified