Introduction

Electroanalytical techniques conform to most of the principles involved use of green analytical chemistry, such as prevention of a large volume of analytical waste, safer solvents and auxiliaries, elimination of toxic reagents, design for energy efficiency, direct and in situ measurements, avoidance of sample treatment, and reduction of derivatization.1 One of the important objectives of electroanalytical chemistry has been the development of novel non-toxic electrode materials with exceptional properties for multiple applications. Among electrochemical platforms that can serve as convenient replacements for the toxic mercury electrode, the boron-doped diamond electrode (BDDE) has attracted considerable attention.2 The BDDE possesses environmentally favorable features and exhibits several interesting electroanalytical attributes, for instance low and stable background current over a wide potential range, corrosion resistance, high thermal conductivity and high current densities, low sensitivity to dissolved oxygen, resistance to fouling because of weak adsorption of polar species on the H- and O-terminated surface, and good responsiveness for many redox analytes without pretreatment.3–7 The electrochemical sensing is an essential technique in the analysis of pharmaceuticals.8 Febuxostat (FBX) is a novel non-purine selective xanthine oxidase inhibitor (inset of Fig. 1) used in the treatment of hyperuricemia and chronic gout.9 Gout is the most common form of inflammatory arthritis associated with the treatment of hyperuricemia and chronic gout involving elevated serum uric acid levels and deposition of monosodium urate crystals in joints and soft tissues. Control of serum uric acid levels is also very important during chemotherapy in patients with malignant tumors for prevention of tumor lysis syndrome.10 FBX inhibition is more potent than allopurinol at the doses used commonly. Furthermore, FBX has multiple excretion pathways and its pharmacokinetics is not greatly dependent on renal clearance, contrary to allopurinol, which may be an advantage in patients with chronic kidney disease and renal insufficiency. The evidence on its use in transplant patients has been also recently

Green Electroanalytical Method for Fast Measurement of Xanthine Oxidase Inhibitor Febuxostat

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The electrooxidation of xanthine oxidase inhibitor febuxostat was investigated on a boron-doped diamond electrode in aqueous solution. The oxidation of the drug molecule was irreversible and exhibited a diffusion-controlled form. A green electroanalytical method for simple and fast measurements of febuxostat was developed at the unmodified electrode surface. The analyses were performed using square-wave voltammetric peak current at 1.38 V. The linear response was obtained in the range of 7.5 \( \times \) 10^{-7} – 2.0 \( \times \) 10^{-5} M with the detection limit of 9.5 \( \times \) 10^{-8} M. The practical analytical value of the method is demonstrated by quantitative determination of febuxostat in film-coated tablets with excellent recovery of 99.6%. Interference studies reveal that uric acid shows a well-developed voltammetric response at +0.64 V. In view of this, the electroanalytical performances of the boron-doped diamond electrode can open up new possibilities for development of the method for simultaneous clinical analysis of febuxostat and uric acid.

Keywords Febuxostat, boron-doped diamond electrode, voltammetry, green electrochemistry, determination

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released. Prevalence of gout and/or hyperuricemia during the past 10 years has been increasing due to an increase of two important risk factors for this disease, obesity and aging. Due to long-term therapy, efficient analytical methods are required to quantify FBX and to evaluate the quality of its pharmaceutical formulations.

An official method for quantification of FBX in bulk form and pharmaceutical formulations has not been approved in any pharmacopoeia. Only a few analytical methods have been described for the determination of FBX, including LC-MS methods for pharmacokinetic applications, HPLC methods with UV detection applied in bioequivalence studies, stability-indicating MEKC and RP-HPLC methods for quantification in pharmaceutical formulation, fluorospectroscopy, HPTLC and spectrophotometry. Compared with those methods, electrochemical techniques have several advantages such as simplicity, low-cost, short analysis time and high sensitivity. In spite of that, only three methods have been published on the voltammetric determination of FBX. Habib et al. described quantification of FBX in pharmaceutical formulations by differential-pulse voltammetric method using a hanging mercury electrode. However, the mercury electrodes are considered undesirable due to the toxic character of mercury and related salts. Jain and Sinha investigated electrocatalytic reduction of FBX in methanol at multi-walled carbon nanotubes/aluminium titanate modified glassy carbon electrode. The cathodic peak current at –1.45 V was used for drug quantification. In this method, methanol was used as the toxic solvent. Very recently, the same authors developed a graphene/zinc oxide nanoflowers modified glassy carbon electrode for determination of FBX. The method is also based on reduction of the drug molecule at very negative potential of –1.65 V versus Ag/AgCl in background electrolyte with added toxic methanol. Moreover, both modifications of electrode surface were complicated and time-consuming. Up to date, no attempt has been made to study the electrooxidation behavior of FBX. Therefore, the purpose of this study was to develop a simple electroanalytical method for the rapid and simple determination of FBX based on drug oxidation at a BDDE without any pretreatment and modification of the electrode surface. The excellent mechanical and electrochemical properties of the BDDE make this platform an ideal choice for direct and low-cost measurement of FBX. The practical use of the developed square-wave voltammetric method was demonstrated via high-speed measuring of pharmaceutical dosage forms with no need of any time-consuming sample treatment procedure prior to the active ingredient analysis.

### Experimental

#### Chemicals

FBX, uric acid, ketoprofen and diclofenac sodium were supplied by Sigma-Aldrich (Steinheim, Germany). Ibuprofen was obtained from Fluka (Laramie, USA). 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). Adenuric® (Berlin-Chemie AG, Berlin, Germany) film-coated tablets containing 120 mg of FBX were supplied by a local pharmacy.

A stock solution of FBX was prepared in ethanol at a concentration of 1.0 × 10⁻³ M and stored in the dark under refrigeration. The standard solutions were prepared daily by dilution of the stock solution with the selected supporting electrolyte, namely Britton-Robinson (BR) buffer (0.04 in each of acetic, phosphoric and boric acids) adjusted to the desired pH with 0.2 M sodium hydroxide solution (pH 4.5 – 8), acetate buffer (0.1 M CH₃COOH and 0.1 M CH₃COONa, pH 5) and phosphate buffer (0.2 M Na₂HPO₄ and 0.2 M NaHPO₄, pH 5.7).

#### Instrumentation and procedures

Voltammetric experiments were performed on a µ-Autolab potentiostat (Eco Chemie, Utrecht, the Netherlands). The experimental conditions were controlled by General Purpose Electrochemical System (GPES) software, Ver. 4.9. A three-electrode cell system was used with a BDDE (Windsor Scientific Ltd., United Kingdom; 3-mm diameter disc) as working electrode, an Ag/AgCl (KCl 3 M, Metrohm) reference electrode and a platinum counter electrode. All measurements were realized at room temperature (23 ± 2°C). Prior to use, the BDDE was cleaned by rinsing with deionized water and gently rubbed on a piece of filter paper until a mirror-like surface was observed. Then, the BDDE was anodic-activated by applying +2 V for duration of 90 s in 0.5 M H₂SO₄ to obtain a predominantly O-terminated electrode surface. Cyclic voltammetry was carried out from 0 to 1.7 V with the scan rate varying from 25 to 3000 mV s⁻¹. For analytical application, square-wave voltammograms were recorded from 0.8 to 1.6 V. The following parameters were employed for square-wave voltammetry (SWV): pulse amplitude of 50 mV, frequency of 300 Hz, and scan increment of 4 mV.

#### Analysis of film-coated FBX tablets

Ten film-coated tablets of FBX were weighed and crushed in a mortar. An amount of prepared powder was weighted to prepare a stock solution of FBX concentration 1.0 × 10⁻³ M and dispersed in ethanol in a 10.0-mL calibrated flask. The mixture was sonicated for 5 min to obtain complete dissolution of the active ingredient and filtered through 0.45 µm Acrodisc GHP filters (Gelman, Ann Arbor, USA). The sample was diluted with BR buffer pH 5 to obtain final concentrations in the range of the calibration curve and subjected to direct SWV measurements. The nominal content of FBX in the formulated product was determined at the BDDE by standard addition method.

### Results and Discussion

#### Electrochemical oxidation of FBX on BDDE

The nature of the FBX oxidation process was studied by cyclic voltammetry in a wide electrochemical potential window at the BDDE without surface modification. As shown in the inset of Fig. 1, FBX was oxidized on the BDDE producing only one well-defined anodic peak in BR buffer pH 5 at 1.38 V in comparison with a glassy carbon electrode (GCE). The oxidation of the FBX molecule appeared as the irreversible process suppressed in following successive cyclic scans due to passivity of the electrode surface (Fig. 1). The irreversibility leads to the absence of any reduction peak in the reverse scan, even if the scan rate is increased up to 3000 mV s⁻¹ (Fig. 2). The oxidation peak current of FBX increased linearly with the square root of the scan rate in the range of 25 – 3000 mV s⁻¹ (inset a of Fig. 2). The fitted regression equations can be expressed as follows: \( \log i_0(\mu A) = 0.345 \log v (mV s^{-1}) - 1.267 \) with a correlation coefficient of 0.998 \((n = 17)\), showing the characteristics expected for the diffusion-controlled electrode process. As can be seen in inset b of Fig. 2, the plot of the logarithm of peak current versus logarithm of scan rate gave a straight-line following the equation: \( \log i_0(\mu A) = 0.621 \log v (mV s^{-1}) - 0.891 \) (correlation coefficient 0.999; \(n = 17\)).
The slope value is very close to the theoretical value of 0.5 expected for an ideal reaction of solution species. As the scan rate increased, the oxidation peak potentials shifted to more positive potentials. The peak potential depends linearly with the logarithm of the scan rate with a slope of 41.9 mV/decade. Using the value of the charge transfer coefficient obtained from the difference between the peak potential ($E_p$) and the half peak potential ($E_{p/2}$) using the equation

$$\Delta E_p = E_p - E_{p/2} = \frac{47.7}{\alpha} \text{ mV}$$

for irreversible electrochemical reaction, the number of electrons exchanged was calculated to be $n = 1.18$. The effect of electrolyte pH on the oxidation reaction of FBX at the BDDE was explored by SWV (Fig. 3). The peak potential of FBX voltammetric response is independent of pH value in the investigated range from 4.5 to 8 with corresponding expression of $E_p(V) = 1.43 - 0.008pH$ (inset a of Fig. 3), showing that the redox reaction involves a one-electron transfer without participation of any proton in the rate determination step. Considering the molecular structure of FBX, thiazole moiety may show an electrooxidation response on the BDDE in which the drug molecule loses an electron to form a cation radical. However, the complete electrooxidation pathway for the FBX molecule should be subjected for further mechanistic studies.

The pH value of the background electrolyte is an important parameter for the electroanalytical measurements due to its influence on the current response. The electrochemical oxidation of FBX at the BDDE was examined in different supporting electrolytes, such as acetate, phosphate and BR buffers with or without the addition of ethanol. Analyzing the voltammetric response, the measurements showed that the best-defined peak was obtained in BR buffer without addition of organic solvent. The pH effect on the SWV peak current of FBX was studied (Fig. 4). As seen in the inset of Fig. 4, the linear calibration graph was obtained within the concentration range of $7.5 \times 10^{-7} - 2.0 \times 10^{-5}$ M with regression equation $i_p(\mu A) = 7.23 \times 10^5 c(M) - 0.19$ (correlation coefficient of 0.999). The detection limit (LOD) and quantitation limit (LOQ) of the procedure were calculated to be $9.5 \times 10^{-8}$ M and $2.9 \times 10^{-7}$ M, respectively. LOD and LOQ values were obtained according to the 3 and 10 $s/a$ criterions, respectively, where $s$ is the standard deviation of the current response of blank solution (five runs) and $a$ is the slope of the related calibration graph. The replicate measurements of $7.5 \times 10^{-6}$ M FBX solution at the BDDE, with simple cleaning step between each voltammetric scan, exhibited good repeatability with a calculated relative...
unaffected by the presence of 100-fold concentration of uric acid (line a) and FBX (line b) recorded at the BDDE in BR buffer pH 5. Concentration of FBX, 1.0 \times 10^{-5} \text{ M}; SWV settings same as in Fig. 3. Inset: voltammetric responses of film-coated tablet solution for increasing drug concentration.

Prior to the analysis of pharmaceutical samples, various possible interferents were tested by analyzing a standard solution of 1.0 \times 10^{-5} \text{ M FBX} (Supporting Information). The tolerable limit was taken as a relative error less than \pm 5\% in the determination of the FBX. According to the analysis of the obtained FBX response at the BDDE, glucose, lactose, citric acid (300-fold), dopamine (100-fold), and folic acid (25-fold) did not show interference in the determination of FBX. In the presence of ascorbic acid, a new well-defined oxidation peak appeared at the potential +0.81 V. However, the results showed that a 20-fold concentration of ascorbic acid had no influence on the peak current of FBX. Selectivity of the developed procedure for FBX measurement was investigated by observing any interference encountered from nonsteroidal anti-inflammatory drugs (NSAIDs) commonly used in the acute treatment of gout. Equal concentrations of diclofenac, ibuprofen and ketoprofen did not cause any change in the FBX current. In the presence of ascorbic acid, a new oxidation peak was found in the voltammogram at +0.83 V. The difference between the peak potentials of FBX and uric acid, indicating that the electroanalytical performance of the BDDE could be used to develop the method for simultaneous clinical analysis of FBX and uric acid whose oxidation potential was shifted +740 mV relative to FBX. The amount of uric acid in the human body has great clinical values in the diagnosis and treatment of gout and hyperuricemia. The expected concentration of FBX in serum samples is 2.8 \mu g/mL (8.85 \times 10^{-6} \text{ M}) after the treatment with therapeutic daily doses of 80 mg,\textsuperscript{29} while the uric acid concentration found in serum of patients with hyperuricemia and chronic gout is about 0.68 mg/L (3.5 \times 10^{-5} \text{ M}).\textsuperscript{30}

**Sample analysis**

To verify the practicality of the developed method, film-coated FBX tablets were analyzed using the standard addition method (inset of Fig. 5). The values obtained using the BDDE were very close to those indicated by the producer. The analysis of FBX in its pharmaceutical formulation exhibited an average recovery of 99.6\%. Furthermore, the RSD of 2.3\% was achieved. To evaluate possible interactions with excipients, recovery experiments were carried out by spiking the formulation solution samples with known amounts of standard FBX solution. The mean recovery of 98.9\% indicated that excipients have no interference effect on the analysis of active ingredients. The time-consuming separation step is avoided, while the SWV scan requires only 0.66 s using the pulse frequency of 300 Hz with 4 mV potential step. The excellent percentage recoveries and reduced values of RSD imply that the proposed method could be applicable for the analysis of formulation products containing FBX.

**Comparison of BDDE with other reported methods for FBX determination**

The green electroanalytical method developed using a BDDE was compared with previously published electrochemical methods based on drug reduction (Table 1). The wide linear range obtained at the BDDE is comparable to those found using modified electrodes.\textsuperscript{26,27} In addition, the BDDE has a wider linear range than the previously reported toxic mercury electrode.\textsuperscript{29} Although the LOD value achieved with the BDDE is in a higher concentration, the working range of the presented method is satisfactory for quantification of FBX in pharmaceutical products. Moreover, the measurements can be obtained more quickly without the need for the addition of toxic solvent to the supporting electrolyte and the use of a BDDE is simpler, as it does not require time-consuming and complicated procedures for electrode surface modification before voltammetric scan. The comparison between the green method developed at the BDDE and other analytical methods described in the literature for FBX determination was also performed. The linearity range and the LOD value obtained at the BDDE are comparable to those reported for some chromatographic methods\textsuperscript{18,19,20} and capillary electrophoresis.\textsuperscript{18} Therefore, the developed electroanalytical method at the BDDE is practically useful for the assay of FBX in pharmaceutical samples, especially due to the extremely short analysis time and low running cost.

**Table 1** Comparison of analytical performance of the BDDE with previously reported modified electrodes for FBX determination

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Concentration range/M</th>
<th>LOD/M</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>Hanging mercury electrode</td>
<td>3.9 \times 10^{-6} – 6.3 \times 10^{-7}</td>
<td>2.1 \times 10^{-8}</td>
<td>21</td>
</tr>
<tr>
<td>MWCNT/Al titanate GCE</td>
<td>1.6 \times 10^{-7} – 3.2 \times 10^{-8}</td>
<td>8.1 \times 10^{-9}</td>
<td>22</td>
</tr>
<tr>
<td>Graphene/ZnO nanoflowers</td>
<td>3.1 \times 10^{-5} – 1.3 \times 10^{-4}</td>
<td>0.5 \times 10^{-5}</td>
<td>23</td>
</tr>
<tr>
<td>GCE</td>
<td>7.5 \times 10^{-7} – 2.0 \times 10^{-4}</td>
<td>9.5 \times 10^{-8} This work</td>
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Conclusions

The paper describes, for the first time, the oxidative behavior of FBX and its electrochemical determination using an unmodified BDDE. This green analytical method delivers high sensitivity, quickness and good reproducibility. It is successfully applied to determine FBX in pharmaceutical formulations and highly recommended for laboratory quality control. The analytical response of FBX at the BDDE is unaffected in the presence of uric acid. Therefore, this finding opens the possibility of developing new methods for the simultaneous analysis of both compounds in a very short analysis time.

Supporting Information

Influence of potential interfering substances on the SWV response of FBX are shown in Supporting Information. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

References