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# Adsorptive anodic stripping differential pulse voltammetric determination of CellCept at $Fe_3O_4$ nanoparticles decorated multi-walled carbon nanotubes modified glassy carbon electrode

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ABSTRACT

A simple and sensitive method based on adsorptive anodic stripping differential pulse voltammetry (AASDPV) for the determination of cellcept, using a magnetic $Fe_3O_4$ nanoparticles and functionalized (carboxylated) multi-walled carbon nanotubes modified glassy carbon electrode (f-MWCNs/$Fe_3O_4$/GCE) was developed. In phosphate buffer solution (pH = 5), the voltammogram of cellcept exhibited tow anodic peaks and the well-defined peak at about 0.611 V vs SCE was used for its monitoring. The modified electrode was characterized by different methods such as electrochemical impedance spectroscopy (EIS), scanning electron microscopy (SEM) and cyclic voltammetry (CV). The experimental parameters, such as pH, deposition potential and time, as well as scan rate were optimized. Under the optimized conditions, $I_p$ (μA) was proportional to the cellcept concentration in the range of 0.05 −200 μM ($R^2$ = 0.9989) with a detection limit of 9.0 nM and limit of quantification of 30.2 nM. The recovery was >98%. The practical analytical utilities of the modified electrode were demonstrated by the determination of cellcept in human urine and blood serum samples. Modified electrode showed an adequate sensitivity and stability for evaluated samples.



## 1. Introduction

CellCept® (mycophenolate mofetil, MMF) chemically known as 2-morpholinoethyl (E)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate [1] is an immunosuppressive agent (Scheme 1).

MMF is rapidly absorbed following oral administration and hydrolyzed to its metabolite, mycophenolic acid (MPA). MPA as a newer immunosuppressant is mainly used in acute rejection treatment [2–4]. It is clear that MMF has potential use in a number of immunological disorders because of its relatively benign side effect profile and observed efficacy [5,6]. MMF is a prodrug of mycophenolic acid that exhibits its effect through inhibition of inosine monophosphate dehydrogenase (IMPDH). IMPDH is the rate-limiting enzyme in the novo synthesis of guanosine nucleotides. T and B lymphocytes are highly dependent on the *de novo* pathway for purine synthesis; however, other cell types can use the salvage pathway for the generation of purine. Moreover, MPA is a fivefold more potent inhibitor of the type II isoform of IMPDH, which is expressed in activated lymphocytes, than of the type I isoform of IMPDH, which is expressed in most cell types. MPA has therefore a more potent cytostatic effect on lymphocytes than on other cell types. This is the principal mechanism by which MPA exerts immunosuppressive effects [7–10]. MMF is used for the prophylaxis of graft rejection in thoracic, small bowel, liver [11], lung [11,12], and pancreas transplantation [9].

Different methods such as high performance liquid chromatography (HPLC) [13–16], liquid chromatography-mass spectrometry (LC-MS) [17], solid phase extraction-reverse phase-high performance liquid chromatography (SPE-RP-HPLC) [18], spectrophotometry [19,20] and micellar electrokinetic chromatography [21] have been reported for the determination of MMF and MPA in bulk and real biological samples. The chromatographic and spectroscopic methods are tedious and time consuming. Literature survey revealed that just two electrochemical methods were reported up to now for the determination of MMF in bulk drug, formulations and biological samples [22,23]. This prompted us to develop a simple, rapid, sensitive, selective and cheap analytical method for routine analysis of MMF.

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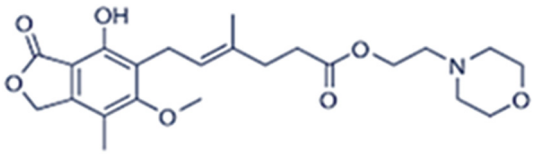

**Scheme 1.** Chemical structure of mycophenolate mofetil.

In recent years, magnetic nanoparticles (MNPs) as a kind of new functional material have become one of the hot favorite for research. MNPs have different chemical, electrical, optical and magnetic properties than their bulk size [24–26] because they are nanoparticles composed of magnetic elements, particularly iron oxide and have large surface-to-volume ratio owing to their small size provides them with a high immobilization density and high surface reactivity [27–29]. Furthermore, due to adsorption ability of magnetic iron oxide and its low-cost, environmentally friendly and easy-prepared material, it has been used as modifier in electrochemical sensor construction [30,31]. Electrochemical sensing and spectrophotometric detection based on the modification or use of a particular variety of inorganic nanoparticles constitute a fascinating research area in analytical applications.

Carbon nanotubes (CNTs) have a wide application in electrochemical sensors [32,33] and biosensors [34,35] since they possess unique properties [32,36,37], such as high electrical conductivity, outstanding catalytic activities, high adsorptive property, excellent biocompatibility, modifiable sidewall, and high reactivity. Thus make CNTs alone or their composite film with other materials such as conducting polymers, ceramics, metal oxides etc are very attractive materials for the development of electrochemical sensors. Of particular interest are CNTs with magnetic properties because of their potential applications in biological labeling, drug delivery, magnetic storage media, separation and electrochemical sensor [27,28,38–41]. The use of magnetic fields to align CNTs [42] and to self-assembleCNT devices [43] has been reported. Recently, Musameh and Wang have demonstrated that the magnetic and catalytic properties of CNTs can be exploited for the magneto-switchable control of electron-transfer reactions [44].

The propose the present work is construction of a voltammetric MMF sensor thoroughly based upon casting of functionalized (carboxylated) multi-walled carbon nanotubes/magnetic $Fe_3O_4$ nanoparticles (f-MWCNs/$Fe_3O_4$) composite at the glassy carbon electrode. The electrochemical characteristic of modified glassy carbon electrode was investigated by electrochemical impedance spectroscopy (EIS) and cyclic voltammetry and its morphology was also studied by scanning electron microscopy (SEM). The analytical performance of the f-MWCNs/$Fe_3O_4$/GC modified electrode was then evaluated with respect to detection limit, linearity, stability and reproducibility. To our knowledge, this is a first demonstration with the combination of magnetic iron oxide and f-MWCNTs to achieve high selective and sensitive voltammteric method for MMF detection using differential pulse anodic stripping voltammetry technique in biological samples.

## 2. Experimental

### 2.1. Apparatus

Voltammetric measurements were carried out with an Autolab (Eco Chemie B. V. Netherlands) controlled by the NOVA software (Version 1.11). A conventional three-electrode cell equipped with a SCE as reference electrode, a platinum wire as auxiliary electrode and f-MWCNTs/$Fe_3O_4$ modified glassy carbon electrode or bare GCE as working electrode was used and measurements were carried out at ambient temperature. A Metrohm pH-meter (model 691) was also applied for pH adjustment. Scanning electron microscopy (SEM) images were obtained with a field emission gun scanning electron microscope (Philips XL 30, USA).

### 2.2. Chemicals and reagents

All solutions were prepared with deionized distilled water. MMF was purchased from Zahravi Pharmaceutical Company (Tabriz, Iran) and used without further purification. MMF stock solution ($1.0 \times 10^{-3}$ M) was prepared in a 1:1 (v/v) mixture of water and ethanol and stored in a refrigerator at 4 °C. Multi walled carbon nanotubes (MWCNTs) with purity 95% (10 nm diameters) and 1–2 μm length was obtained from Nanolab (Brighton, MA). All other chemicals were of analytical reagent grade and used without further purification. The supporting electrolyte for the electrochemical investigations was 0.1 M phosphate buffer solution (PBS) with different pH which were prepared by mixing the stock solutions of $Na_2HPO_4$ and $NaH_2PO_4$. All the experiments were carried out at constant temperature (25 C).

### 2.3. f-MWCNTs/$Fe_3O_4$ composite preparation

f-MWCNTs/$Fe_3O_4$ composite was synthesized by chemical coprecipitation according to the reported method with a minor modification [45–47]. In brief, 0.1 g of MWCNTs was added to a solution containing of concentrated nitric acid (1 mL) and hydro chloric acid (3 mL) and sonicated for 12 h. After addition of 10 mL of distilled water to the resulted mixture, it was centrifuged; the residual solid was washed four times with distilled water and then dried under vacuum at 50 °C overnight. Then 0.05 g of the product (f- MWCNTs) was dispersed in 20 mL of distilled water in an ultrasonic bath for 20 min and then 0.35 g of $FeCl_3 \cdot 6H_2O$ was added. After 30 min vigorous stirring under $N_2$ atmosphere, 0.065 g of $FeCl_2 \cdot 4H_2O$ was added to the resulted dispersed solution and continued stirring under $N_2$ atmosphere for 30 min. The 0.5 M NaOH solution was used for adjustment of pH of coprecipitation reaction. $N_2$ was used during the reaction to prevent critical oxidation. Black composite obtained from the previous stage was collected by sedimentation with a help of an external magnetic field and washed several times with doubly distilled water until stable ferrofluid was obtained. Eventually, the f-MWCNTs/$Fe_3O_4$ composite was dried under vacuum at 50° C overnight. A simplified graphical representation of f-MWCNTs/$Fe_3O_4$ preparation is illustrated in Scheme 2.

### 2.4. Fabrication of the f-MWCNTs/$Fe_3O_4$/GCE

Prior to modification, the bare glassy carbon electrode (3 mm diameter) was polished to a mirror-like surface with 0.05 μm alumina slurry and sonicated in a 1:1 (v/v) mixture of water and ethanol for 3 min and dried at room temperature. The polished electrode was then electrochemically activated by potential cycling between −1.0 and 1.0 V in 0.5 M $H_2SO_4$ until a stable cyclic voltammogram was obtained. 10 mg of f-MWCNTs/$Fe_3O_4$ composite were dispersed in 10 mL dimethylformamide (DMF) and homogenized under sonication for 30 min and then 4 μl of suspension was dropped onto the surface of the cleaned bare glassy carbon electrode and dried in air at room temperature. Finally, the modified electrode was immersed in phosphate buffer solution of pH 5.0 and cycled by several successive potential scanning from 0.4 to 1.1 V at scan rate of 100 mV/s until a steady voltammogram was obtained.