J Appl Electrochem (2009) 39:2375–2384
DOI 10.1007/s10800-009-9924-0

ORIGINAL PAPER

Utilization of a montmorillonite-Ca-modified carbon paste electrode for the stripping voltammetric determination of diflunisal in its pharmaceutical formulations and human blood

A. M. Beltagi

Received: 20 January 2009 / Accepted: 29 April 2009 / Published online: 22 May 2009

Abstract A highly sensitive square-wave adsorptive anodic stripping voltammetric method was described for the determination of diflunisal in its formulations and human blood, utilizing a developed montmorillonite-Ca-modified carbon paste electrode (CPE). The peak current was significantly enhanced due to the strong adsorptive properties of montmorillonite-Ca clay. The optimal procedural parameters were frequency $f = 80$ Hz, scan increment $\Delta E_a = 10$ mV, pulse-amplitude $\Delta E_i = 25$ mV, and an accumulation potential E_{acc} of 0.0 V versus Ag/AgCl/3M KCl in acetate buffer of pH 5.0 using 10% (w/w) MMT-Ca-modified CPE. The described method was successfully applied for assay of diflunisal in different pharmaceutical formulations (Doloban®, Dolozal®, and Maxipan® tablets) with mean percentage recoveries of 98.72 ± 0.35, 99.24 ± 0.89, and 98.20 ± 1.38, respectively. Furthermore, the method was successfully applied for assay of diflunisal in spiked human serum without the necessity of sample pretreatment or time-consuming extraction prior to the analysis. Mean percentage recovery of diflunisal in human serum was 99.16 ± 1.03 with a limit of detection of 3.0×10^{-9} M (0.75 ng mL^{-1}). Due to this extremely low limit of detection, the proposed method was used to follow up the concentration of drug in blood samples of two male volunteers after oral administration of a single dose of Dolozal®, 500 mg tablet.

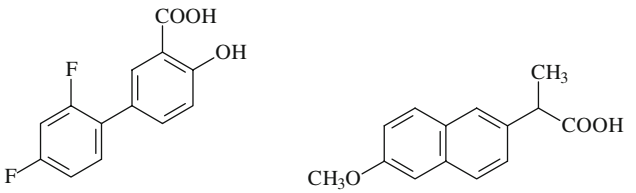
Keywords Diflunisal · Montmorillonite-Ca · Square-wave voltammetry · Tablet analysis · Human serum · Pharmacokinetic

A. M. Beltagi (✉)
Chemistry & Physics Department, Faculty of Education, Kafr El-Sheikh University, 33616 Kafr El-Sheikh, Egypt
e-mail: ambeltagi@hotmail.com

1 Introduction

Diflunisal [DIF, 5-(2,4-difluorophenyl)salicylic acid, Scheme 1] is a synthetic difluorophenyl derivative of salicylic acid with similar analgesic and anti-inflammatory properties to aspirin. It is used to treat mild to moderate pain and relieve the inflammation, swelling, stiffness, and joint pain associated with rheumatoid arthritis and osteoarthritis [1]. A comparison of the pharmacological profile of DIF with those of some well-known non-steroidal anti-inflammatory agents such as aspirin, ibuprofen, and indomethacin showed that DIF is more potent and less toxic than these drugs [2]. DIF is rapidly and completely absorbed following oral administration and the maximum plasma concentration occurring between 2 and 3 h. More than 99% of DIF in plasma is bound to proteins. Peak plasma concentrations C_{max} of 87 ± 17 μg mL^{-1} with an elimination half-life time k_{el} of 8–12 h were observed following a single 500 mg dose (http://www.Drugs.com/pro/diflunisal.html). DIF is almost completely eliminated by Phase II metabolism: formation of the acyl glucuronide (40–50% of the dose), the phenolic glucuronide (30–40%), and the sulfate conjugates (10–20%) usually accounting for more than 90% of an oral dose [3]. The conjugates of DIF are eliminated by renal excretion mainly by active tubular secretion [4]. Little or no DIF is excreted in the feces. It appears in human milk in concentrations of 2–7% of those in plasma. DIF is usually available in its pharmaceutical formulations individually or as binary mixtures with naproxin [Nap, (+)-2-(6-methoxy-2-naphthyl) propionic acid, Scheme 1], a non-steroidal anti-inflammatory drug with the same analgesic and anti-inflammatory properties of DIF.

Montmorillonite belongs to the smectite group of clays with a layer lattice and includes two types: montmorillonite sodium (MMT-Na) and montmorillonite calcium



Diflunisal Naproxen

Scheme 1 Chemical structure of diflunisal and naproxin

(MMT-Ca). Because montmorillonite has high chemical and mechanical stability, a well-layered structure and strong adsorptive properties attributed to the expandability of the internal layers, it has been successfully used in electroanalytical chemistry as a modifier in carbon electrodes for assay of different organic [5–7] and inorganic [8–12] species.

Several methods have been reported for the assay of DIF in its formulations and biological fluids. These methods employed spectrophotometry [13–15], spectrofluorimetry [16–22], capillary electrophoresis [23, 24], liquid chromatography (LC) [25–27], gas chromatography coupled with mass spectrometry (GC/MS) [28], and high performance liquid chromatography (HPLC) [29–33] techniques. Moreover, DIF is early determined in its tablets at mercury electrode using differential pulse polarographic (DPP) and differential pulse adsorptive stripping voltammetric (DP-AdSV) techniques with limits of detection (LOD) of 5.0 and 0.1 μg mL^{-1}, respectively [34].

The main objective of the current study is to develop a sensitive and convenient electroanalytical method for the determination of DIF in formulations and biological fluids, utilizing the excellent properties of montmorillonite-calcium (MMT-Ca)-modified carbon paste electrode (CPE).

2 Experimental

2.1 Apparatus

All voltammetric measurements were performed with Princeton Applied Research (PAR, Oak Ridge, TN, USA) Potentiostats Models 263A and 273A. A voltammetric cell consisting of a C-2 stand with a carbon paste working electrode body (BAS Model MF-2010, 3 mm in diameter and 1 mm in depth), an Ag/AgCl/3M KCl reference electrode (BAS Model MF-2063), and a platinum wire counter electrode (BAS Model MW-1032) was used. A magnetic stirrer (PAR-305) with a Teflon-coated magnet was used to provide the convective transport during the preconcentration step. The data were treated through a personal computer connected to the potentiostat and loaded with the 270/250 Research Electrochemistry software version 4.41—Copyright© 2000 (PerkinElmer Instruments Inc.).

A Mettler balance (Toledo-AB104, Greifensee, Switzerland) was used for weighing the solid materials. A pH-meter (Crison, Barcelona, Spain) was used for the pH measurements. An Eppendorf centrifuge (Model 5417 C, Hamburg, Germany) was used for separation of the precipitated proteins from human serum samples prior to the assay. A micopipetter (Eppendorf-Multipette® plus) was used for transfer of the analyte solutions throughout the present experimental work. The de-ionized water used throughout the present study was supplied from a Purite-Still Plus de-ionizer connected to an AquaMatic bi-distillation water system (Hamilton Laboratory Glass Ltd, Kent, UK).

2.2 Reagents and solutions

2.2.1 Supporting electrolytes

Britton–Robinson (B–R) universal buffer (pH 2–11), acetate buffer (pH 3.8–6.2) and phosphate buffer (pH 2–7.5) solutions were prepared in de-ionized water and were used as supporting electrolytes. All the chemicals were of analytical grade and were used without further purifications.

2.2.2 Solutions of bulk DIF and NAP

Bulk DIF was kindly supplied from Sigma Pharmaceutical Industries, Egypt. Bulk NAP was kindly supplied from RAMEDA for Pharmaceutical Industries & Diagnostic Reagents, 6th of October City, Egypt. Standard stock solutions of 1×10^{-3} M bulk DIF and NAP were prepared in methanol (Merck) and stored in dark bottles at 4 °C. The desired solutions (1×10^{-6} to 1×10^{-4} M) were prepared by appropriate dilution of the standard stock solutions with methanol.

2.2.3 Interferences

Standard stock solutions of 1×10^{-3} M of each of aspirin (acetylsalicylic acid), Benorilate (4-acetamidophenyl *O*-acetyl salicylate), *O*-acetylsalicylamide, ketoprofen (2-(3-Benzoylphenyl)propionic acid), ketorolac (*RS*-5-Benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid), and ibuprofen (*RS*-2-(4-Isobutylphenyl)propionic acid) were prepared in methanol (Merck) and used as foreign species in the interferences studies.

2.2.4 Solutions of Doloban®, Dolozal®, and Maxipan® tablets

Ten tablets of each of Doloban® (RAMEDA for Pharmaceutical Industries & Diagnostic Reagents, Egypt), Dolozal® (Sigma Pharmaceutical Industries, Egypt), and Maxipan®

(RAMEDA for Pharmaceutical Industries & Diagnostic Reagents, Egypt) as labeled to containing 250 mg DIF, 500 mg DIF, and a binary mixture of 200 mg DIF + 200 mg NAP, respectively, were weighed, and the average mass per tablet was determined. A portion of each of the finely ground tablets was transferred accurately into a 100 mL calibrated flask, contains 70 mL methanol (Merck). The content of each flask was sonicated for about 15 min and then filled up with methanol. The solution was then filtrated through a 0.45 μm milli-pore filter (Gelman, Germany) to separate out the insoluble ingredients, rejecting the first portion of the filtrate. The desired concentrations of the drug were obtained by accurate dilution with methanol.

2.2.5 *Solutions of human serum*

Serum samples of healthy volunteers were stored frozen until assay. Samples of the human serum (each of 1 mL) were fortified with various concentrations (2×10^{-8} to 1×10^{-6} M) of DIF in small tubes. Each of these samples was then completed to 2 mL with methanol to denature and precipitate proteins. After vortexing each of the serum samples for 2 min, the precipitated proteins were separated by centrifugation for 3 min at 14000 rpm. The clear supernatant layer was filtered through a 0.45 μm milli-pore filter to obtain protein-free human serum samples. Then the analysis was followed up as indicated in the general analytical procedure.

2.3 Preparation of the modified CPE

A total of 4.5 g of graphite powder (1–2 μm, Aldrich, Milwaukee, WI, USA) and 0.5 g of montmorillonite-Ca clay (Fine powder < 5 μm, ECC America Inc., Southern Clay Products Subsidiary, Gonzales, Texas, USA) were mixed uniformly by milling in a small agate mortar; then 1.8 mL Nujol oil (Sigma, $d = 0.84$ g mL^{-1}) was added and milled again to give a homogenous MMT-Ca-modified carbon paste (10% w/w). Various modified carbon pastes containing different mass percentages of MMT-Ca clay [0%, 5%, 15%, 20%, and 25% (w/w)] were similarly prepared. An amount of the prepared MMT-Ca-modified carbon paste was pressed into the end cavity (3 mm in diameter, 1 mm in depth) of the electrode body. Surface of the constructed MMT-Ca-modified CPE was manually smoothed by polishing on clean paper before use.

2.4 General analytical procedure

A 10-mL volume of acetate buffer solution (pH = 5.0) was introduced into the micro-electrolysis cell and the smoothed MMT-Ca-modified CPE was then immersed in the supporting electrolyte, and several sweeps were applied to obtain a low background current. After that aliquots of the analyte were introduced into the electrolysis cell, and a selected preconcentration potential was then applied to the developed MMT-Ca-modified CPE for a selected preconcentration time while the solution was stirred at 400 rpm. At the end of the precncentration time, the stirring was stopped and a 5-s rest period was allowed for the solution to become quiescent. The voltammogram was then recorded by scanning the potential toward the positive direction using the square-wave potential waveform. After each measurement, the used modified carbon paste was carefully removed and a new MMT-Ca-modified CPE was constructed as described in Sect. 2.3.

Medium exchange method [35] was used during determination of DIF in the human serum, where the experiment was held after the preconcentration step, and the electrode was then transferred into a blank electrolyte solution to proceed the stripping step. This procedure enables to avoid blocking of the electrode surface with low molecular weight proteins which may remain after centrifugation. Moreover, the perfect choice of the optimal preconcentration potential decreases the possibility of adsorption of the remained low molecular weight proteins at the electrode surface.

In order to study the reproducibility, accuracy, and precision of the described method for determination of DIF, recovery experiments were carried out by means of both the calibration curve and standard addition methods. All the data were obtained at room temperature.

2.5 Pharmacokinetic studies

Two healthy male volunteers with different ages (Subject #1 = 32 years and Subject #2 = 38 years) took part in this study. Subjects were caffeine and alcohol free for at least 12 h before the administration of the drug. The two volunteers gave their written informed consent prior to anticipating in the study (at Ramadan Specialized Hospital, Tanta City, Egypt). Each study subject received a single 500 mg oral dose of DIF (Dolozal® tablet) in the morning, after an overnight fast. Blood samples (1 mL each) were obtained at 0 (predose), 0.5, 1.0, 1.5, 2, 3, 4, 6, 9, 12, and 24 h after the oral administration. The blood samples were centrifuged immediately at 3000 rpm for 15 min and then the plasma fractions were rapidly separated and stored in coded polypropylene tubes at −20 °C until the assay. Following separation of proteins by methanol, the plasma samples were analyzed using the described square-wave stripping voltammetric procedure.

3 Results and discussion

3.1 The electrochemical behavior of DIF at MMT-Ca-modified CPE

The anodic cyclic voltammograms of 1×10^{-6} M DIF in acetate buffer of pH 5.0 were recorded at both bare CPE and MMT-Ca-modified CPE (Fig. 1). It is clear that the oxidation peak current of DIF was significantly enhanced at the MMT-Ca-modified CPE (Fig. 1, curve b) compared to that obtained at the bare CPE (Fig. 1, curve a) under the same experimental conditions. The enhancement of the peak current may be attributed to the strong adsorptive properties of montmorillonite-Ca clay, and subsequently sensitivity of assay of DIF was remarkably improved. Therefore, MMT-Ca-modified CPE was used as a working electrode over the rest of the analytical study.

Cyclic voltammograms of 1×10^{-6} M DIF recorded in Britton–Robinson (pH 2–11), acetate (pH 3.8–6.2), and phosphate (pH 2–7.5) buffer solutions following preconcentration at MMT-Ca-modified CPE for 180 s yielded a single well-defined peak (Fig. 2), corresponding to the oxidation of the –OH group of the analyte (Scheme 1). No reduction peak was observed in the negative scanning half-cycle, indicating the irreversible nature of the electrode process. The peak current magnitude (i_p) was much enhanced in acetate buffer of pH 5.0 (Fig. 2). Therefore, it was used as a supporting electrolyte in the rest of the present analytical work. The peak potential (E_p) shifted to less positive values with the increase of pH, denoting that protons are involved in the electrode reaction process and the proton-transfer reaction precedes the electron transfer process [36]. The plot of E_p versus pH exhibited two linear segments with a break point at a pH of 3. The intersection point observed at pH 3 agrees well with the reported pK_a value of DIF [37].

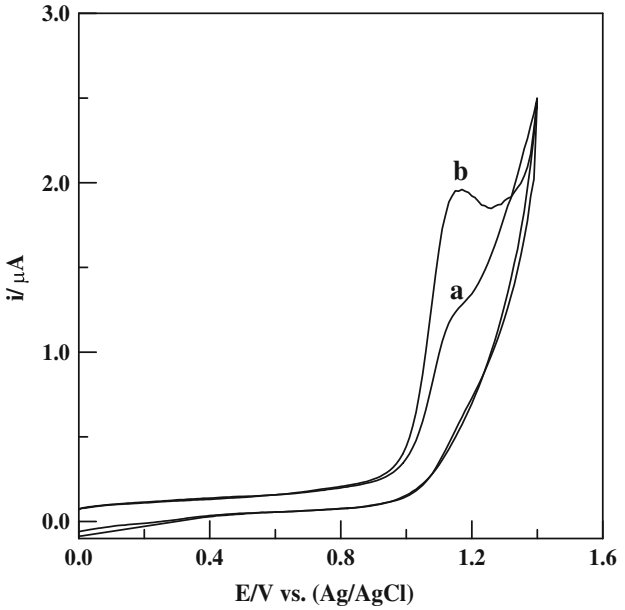


Fig. 1 Cyclic voltammograms of 1×10^{-6} m DIF in acetate buffer of pH 5.0 following its preconcentration at (a) bare CPE and (b) MMT-Ca-modified CPE ($E_{acc} = 0.0$ V, $t_{acc} = 180$ s and scan rate = 0.10 V s^{-1})

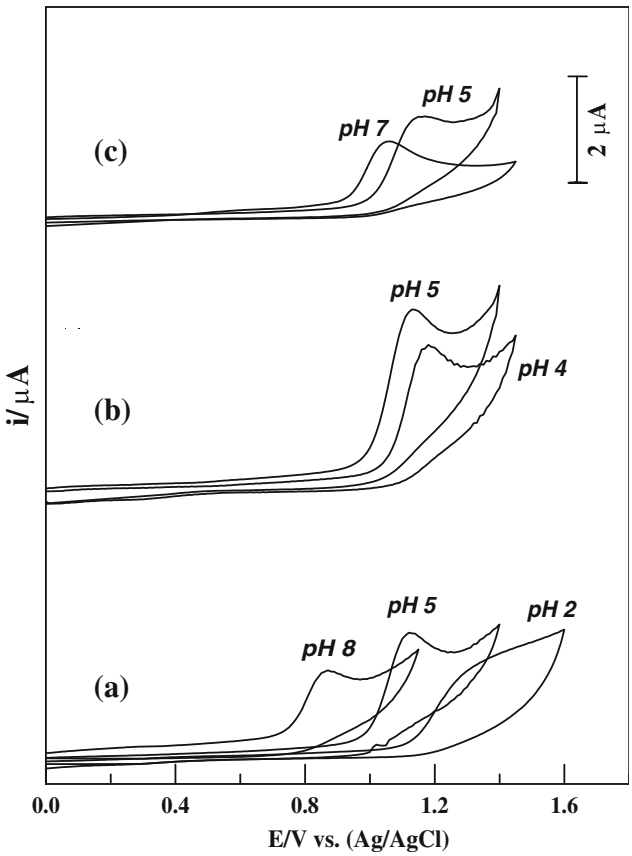


Fig. 2 Cyclic votammograms of 1×10^{-6} M DIF following preconcentration at MMT-Ca-modified CPE by adsorptive accumulation at 0.0 V for 300 s recorded in different supporting electrolytes of various pH values; (a) Britton–Robinson, (b) acetate, and (c) phosphate buffer solutions

The interfacial preconcentration of DIF at the MMT-Ca-modified CPE was designated from cyclic voltammograms of 1×10^{-6} M drug solution recorded in acetate buffer of pH 5.0 following preconcentration under open circuit conditions (Fig. 3, curve a) and repetitive cycles following preconcentration at 0.0 V for 300 s (Fig. 3, curves b and c). The enhancement of the peak current of the first cycle (Fig. 3, curve b) indicated the adsorption of DIF at the electrode surface. A substantial decrease of the monitored voltammetric peak was observed in second cycle (Fig. 3, curve c) indicated rapid desorption of DIF out of the electrode surface. The influence of scan rate (20–300 mV s^{-1}) on either the oxidative peak current (i_p) or peak potential (E_p) was studied. The peak current changed linearly with

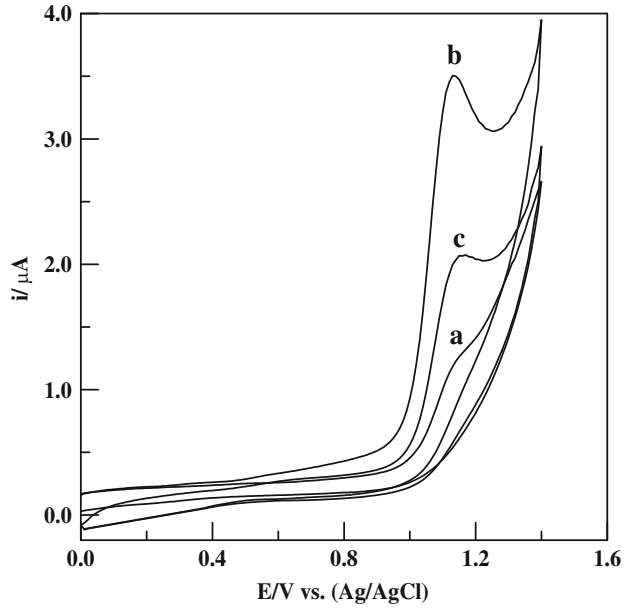


Fig. 3 Cyclic voltammograms of 1×10^{-6} M DIF in acetate buffer of pH 5.0 recorded at scan rate of 0.10 V s^{-1}; (a) at open circuit conditions, (b) following preconcentration at MMT-Ca-modified CPE by adsorptive accumulation at $E_{acc} = 0.0$ V for 300 s, and (c) the repetitive second cycle

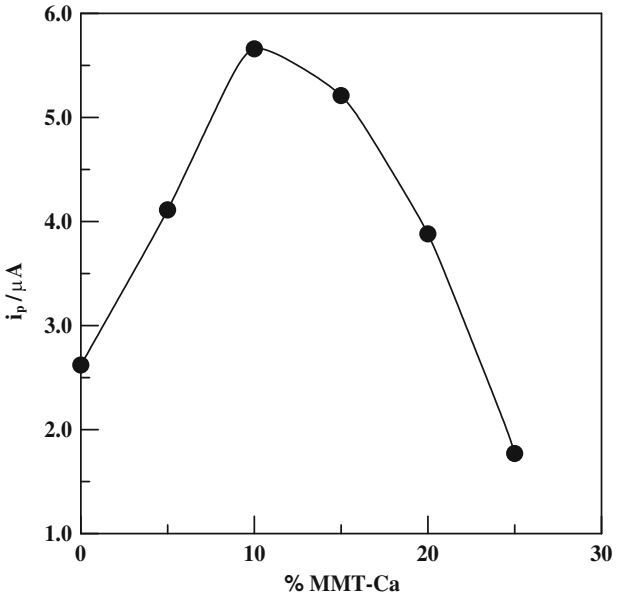


Fig. 4 SW-AdAS voltammetric peak current (i_p) of 1×10^{-7} M DIF as a function of MMT-Ca percentage, recorded in acetate buffer of pH 5.0 following preconcentration by adsorptive accumulation at $E_{acc} = 0.0$ V for 120 s. Instrumental parameters; frequency $f = 60$ Hz, scan increment $\Delta E_i = 10$ mV, and pulse-amplitude $\Delta E_a = 25$ mV

scan rate (v) according to the equation $i_{pa} = Av^x$, where x values is expected as 1.0 or 0.5 for adsorption-controlled or diffusion-controlled reactions, respectively [38]. The regression of log i_{pa} versus log v gave a slope value of 0.92, indicating that the oxidative peak current is of adsorption nature. On the other hand, as scan rate was increased, the peak potential shifted toward more positive potentials as expected for an irreversible oxidation process [39].

3.2 Square-wave voltammetric studies

The strong adsorption phenomenon of DIF at a MMT-Ca-modified CPE was used as an effective preconcentration step prior to the actual stripping voltammetric quantification of the drug. Due to the intense sensitivity of the square-wave mode, it was used in all the analytical study.

3.2.1 Influence of the percentage of MMT-Ca clay

It is clear that MMT-Ca can remarkably improve the square wave adsorptive anodic stripping (SW-AdAS) voltammetry peak current of DIF. However, the percentages of MMT-Ca in the graphite paste of the CPE influence the square-wave anodic stripping peak current of DIF as demonstrated in Fig. 4. The stripping peak current magnitude first increased upon the increase of the percentage of MMT-Ca to a maximum value at 10% MMT-Ca and then decreased. Such enhancement of stripping peak current magnitude is expected due to the strong adsorptive properties of MMT-Ca. However, the conductivity of MMT-Ca-modified CPE will drop when gradually increasing the amount of MMT-Ca, hindering the electron transfer process and increasing the background current. Therefore, a 10% (w/w) MMT-Ca-modified CPE was used in the rest of the present analytical studies.

3.2.2 Square-wave pulse parameters

Since the square-wave response markedly depends on the parameters of the excitement signal, voltammograms of 1×10^{-7} M DIF in acetate buffer of pH 5 following preconcentration at the MMT-Ca-modified CPE for 120 s were recorded at the various instrumental conditions (frequency $f = 20$–140 Hz, scan increment $\Delta E_i = 2$–10 mV, and pulse amplitude $\Delta E_a = 25$–75 mV). The impact of varying the square-wave frequency on the SW-AdS voltammetric peak current magnitude showed that to assure maximum peak current, 80 Hz square-wave frequency was the ideal choice for this operational parameter. In addition, the SW-AdAS voltammetric peak current magnitude showed a linear enhancement when scan increment was varied over the range 2–10 mV. Accordingly, 10 mV scan increment was recommended for the subsequent work. Furthermore, varying the value of excitation wave pulse-amplitude also plays an important role on the measured SW-AdAS voltammetric peak current magnitude. On

increasing the pulse-amplitude over the range 25–75, the monitored peak became broader with higher base line. Therefore, the optimal operational parameters, with respect to the much enhanced peak current magnitude and best peak morphology, could be concluded as: frequency $f = 80$ Hz, scan increment $\Delta E_i = 10$ mV, and pulse-amplitude $\Delta E_a = 25$ mV using acetate buffer of pH 5.0 as a supporting electrolyte.

3.2.3 Preconcentration (accumulation) parameters

Effect of varying the preconcentration potential E_{acc} (−0.20 to +0.50 V vs. Ag/AgCl/3M KCl) on the peak current of the SW-AdAS voltammograms of 1×10^{-7} M DIF in acetate buffer solution of pH 5.0 was evaluated, following preconcentration at the 10% (w/w) MMT-Ca-modified CPE for 120 s (Fig. 5). The results showed that much enhanced peak current magnitude was achieved within the potential range −0.20 to +0.10 V. At more positive preconcentration potential, significant decrease of peak current of DIF was observed. Therefore, a preconcentration potential of 0.0 V was used throughout the present analytical study.

On the other side, SW-AdAS voltammograms of 5×10^{-8} and 1×10^{-7} M DIF were recorded at increase preconcentration time under the foregoing optimal procedural parameters. As shown in Fig. 6, the peak current

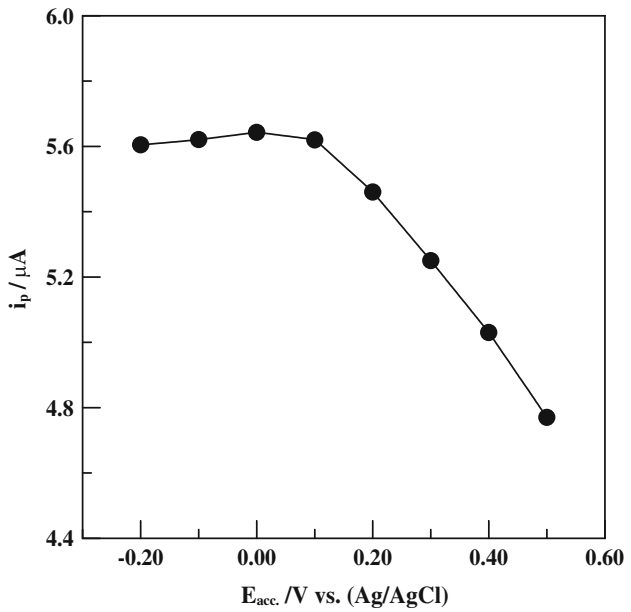


Fig. 5 Effect of accumulation potential (E_{acc}) on the SW-AdAS voltammetric peak current (i_p) of 1×10^{-7} M DIF in acetate buffer of pH 5.0, following preconcentration at 10% MMT-Ca-modified CPE by adsorptive accumulation at $E_{acc} = 0.0$ V for 120 s. Instrumental parameters; frequency $f = 80$ Hz, scan increment $\Delta E_i = 10$ mV, and pulse-amplitude $\Delta E_a = 25$ mV

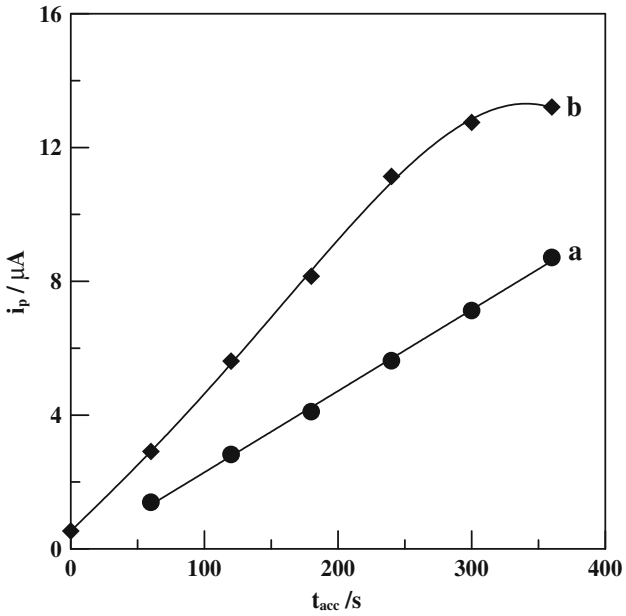


Fig. 6 Effect of the accumulation time (t_{acc}) on the SW-AdAS voltammetric peak current of (a) 5×10^{-8} and (b) 1×10^{-7} M DIF in acetate buffer of pH 5.0 following preconcentration at 10% MMT-Ca-modified CPE by adsorptive accumulation at 0.0 V. Other instrumental parameters are as those given in Fig. 5

magnitude was linearly dependent (before saturation of the electrode surface) on both the analyte concentration and the accumulation time. Apparently, lower concentration of the analyte requires longer preconcentration time. This meant that the choice of preconcentration time was dictated by the sensitivity required. In the present analytical method, preconcentration times of 60, 180, and 300 s were applied (Table 1).

3.3 Method validation

3.3.1 Calibration graph and limit of detection

Under the optimum experimental conditions, good linear correlations were obtained between the SW-AdAS voltammetric peak current of DIF (i_p) and its concentration at different preconcentration time (Table 1). The parameters of the concentration-current straight lines were calculated by the least squares method. The standard curves were linear with correlation coefficients (r) not <0.996 (Table 1), thus confirmed validity of the described SW-AdAS voltammetric method for determination of DIF at the 10% MMT-Ca-modified CPE. Limits of detection (LOD), defined as three times the signal-to-noise ratio (S/N = 3), were evaluated following various preconcentration times (Table 1). The achieved LOD value following preconcentration of the drug at the 10% MMT-Ca-modified CPE for 300 s was 1.5×10^{-9} M (0.375 ng mL^{-1}),

Table 1 Characteristics of the calibration curves of the SWAdAS voltammetric determination of DIF under the optimal procedural conditions

* Average of three determinations

Acc. duration (s)	Linearity range (M)	Least square equation*		Corr. coeff. (r)	LOD (M)
		Intercept (μA)	Slope (μA/μM)		
60	8×10^{-8} to 4×10^{-6}	0.085	38.05	0.997	2.4×10^{-8}
180	3×10^{-8} to 5×10^{-7}	0.112	96.22	0.996	9.0×10^{-9}
300	5×10^{-9} to 2×10^{-7}	0.166	168.51	0.999	1.5×10^{-9}

revealed the high sensitivity of the described SW-AdAS voltammetric method.

3.3.2 Accuracy and precision

Accuracy of the described SW-AdAS voltammetric method was evaluated as recovery of a known concentration (1×10^{-7} M) of DIF following preconcentration by adsorptive accumulation at the 10% MMT-Ca-modified CPE for 120 s. The percentage recovery obtained using the calibration curve method was 99.68% (Table 2). The analytical precision of the developed SW-AdAS voltammetric method was verified from the reproducibility of five replicate determinations of 1×10^{-7} M DIF following preconcentration at the MMT-Ca-modified CPE for 120 s, and the estimated relative standard deviation was 0.61%.

3.3.3 Stability

The stability of DIF in acetate buffer solution of pH 5.0 was evaluated under the optimal operational conditions by monitoring the changes in the anodic peak current of 1×10^{-7} M DIF, following its preconcentration at the 10% MMT-Ca-modified CPE for 120 s over a period of 3 h. The electrochemical signal showed no difference over this time period confirmed stability of DIF in the used supporting electrolyte, even after long periods. Moreover, under storage conditions for more than a month, neither the stock solutions of DIF nor the serum calibration samples showed any degradation.

Table 2 Influence of minor changes in the optimal procedural conditions on assay of 1×10^{-7} M DIF, following its preconcentration at MMT-Ca-modified CPE for 120 s; $f = 80$ Hz, $\Delta E_i = 10$ mV, and $\Delta E_a = 25$ mV

Variable	Conditions	%R ± SD ($n = 5$)
Robustness		
pH of the medium*		
4.5	$E_{acc} = 0.0$ V	99.89 ± 1.03
5.0		99.68 ± 0.61
5.5		99.42 ± 0.73
Accumulation potential (E_{acc})*		
−0.05 V	pH = 5.0	98.68 ± 0.94
0.0 V		99.68 ± 0.61
+0.05 V		100.44 ± 0.73
Intermediate precision		
Potentiostat 263A-PAR (Lab. 1)	pH = 5.0	99.68 ± 0.61
Potentiostat 273A-PAR (Lab. 2)	$E_{acc} = 0.0$ V	97.92 ± 2.26
Day (1)		99.68 ± 0.61
Day (2)		99.10 ± 0.23
Day (3)		98.79 ± 0.90

* Potentiostat 263A–PAR

3.3.4 Robustness and intermediate precision

The robustness [40] of the described SW-AdAS voltammetric method for assay of DIF was examined by evaluating the influence of small variations in some of the most important procedural parameters (pH 5.0 ± 0.5 and accumulation potential E_{acc} 0.0 ± 0.05 V). As shown in Table 2, none of these variables significantly affect the recovery of DIF, which indicated that the described method is robust. The intermediate precision [40] of the described SW-AdAS voltammetric method for assay of DIF at the MMT-Ca-modified CPE was identified using two PAR-Potentiostats, 263A (Lab. 1) and 273A (Lab. 2), at different elapsed time. The results obtained due to Lab.-to-Lab. and even day-to-day were found reproducible (Table 2), since there was no significant difference in the recovery and/or standard deviation values.

3.3.5 Interferences

The competitive co-adsorption interferences were evaluated in the presence of various substances that are usually found in the pharmaceutical formulations. Generally, voltammetric techniques have found widespread use in drug analysis since it is usually involve a simple dilution step and most of the ingredients used do not interfere in the subsequent determination [41]. The selectivity of the described SW-AdAS voltammetric method for the assay of DIF was examined in the presence of the common inactive ingredients usually present in its formulations; croscarmellose sodium, FD&C Blue #2 aluminum lake, hypromellose, microcrystalline cellulose, pregelatinized starch, propylene glycol, sodium stearyl fumarate, and titanium dioxide (http://www.Drugs.com/pro/diflunisal.html). These ingredients are electro-inactive and its adsorption at the MMT-Ca-modified CPE had no significant effect on the

Table 3 Interferences from some foreign species to the assay of 1×10^{-7} M DIF by the described SW-AdAS voltammetric method at the constructed MMT-Ca-modified CPE

Foreign species	Tolerance limit* (M)
Aspirin	5×10^{-5}
Benorilate	5×10^{-5}
O-acetylsalicylamide	4×10^{-5}
Ketoprofen	4×10^{-5}
Ketorolac	3×10^{-5}
Ibuprofen	2×10^{-5}

* For error $<\pm 5\%$

voltammetric signal of DIF under the optimum procedural parameters. Furthermore, binary drug mixtures containing DIF and NAP are increasingly used in the therapy of a variety of diseases. NAP is electro-inactive at the MMT-Ca-modified CPE, since it has no oxidative center (Scheme 1). Therefore, DIF can be determined in the presence of NAP without interference. A mean recovery of DIF in a binary mixture with NAP (1×10^{-7} M DIF + 1×10^{-7} M NAP) and in the presence of ingredients was found to be equal to $99.07 \pm 1.11\%$ ($n = 5$). This means that the described stripping voltammetric method can be considered selective for the assay of DIF, even in the presence of NAP.

Due to the wide spread use of some salicylic acid derivatives as anti-inflammatory and analgesic drugs, the selectivity of the described SW-AdAS voltammetric method for the assay of DIF was evaluated in the presence of aspirin (acetylsalicylic acid), benorilate (4-acetamidophenyl *O*-acetyl salicylate), and *O*-acetylsalicylamide. Since these salicylic acid derivatives are electro-inactive at the constructed MMT-Ca-modified CPE, they showed insignificant interferences to the assay of DIF even at a 500-fold concentration ratio over DIF (Table 3). Moreover, three of the anti-inflammatory and analgesic drugs belonging to different families (ketoprofen, ketorolac, and ibuprofen) were studied as foreign species in 1×10^{-7} M DIF solution, and the results showed also insignificant interference to the assay of DIF (Table 3).

3.4 Analytical applications

3.4.1 Assay of DIF in its formulations

The described SW-AdAS voltammetric method was successfully applied for the determination of DIF in its pharmaceutical formulations (Doloban® 250 mg DIF/tablet, Dolozal® 500 mg DIF/tablet, and Maxipan® 200 mg DIF + 200 mg NAP/tablet). The obtained results (Table 4) were statistically compared with those obtained by a reported spectrophotometric method [15]. Since the calculated value of F does not exceed the theoretical value (Table 4), there was no significant difference between the described voltammetric and the reported spectrophotometric method with respect to reproducibility [42]. Also, no significant difference was noticed between the two methods regarding accuracy and precision as revealed by t-value [42], Table 4. The accuracy of the described SW-AdAS voltammetric method was also judged by applying the standard addition method [43], Table 4. This means that the described voltammetric method should be applicable to the analysis of DIF individually in its formulations and as a binary mixture with NAP. This may be considered a great advantage of the described voltammetric method over most of the reported ones, since assay of DIF in the presence of NAP was difficult due mainly to their similar analytical properties.

3.4.2 Assay of DIF in spiked human serum

The absence of detectable signals in DIF-free serum indicated that the constituents of the biological samples do not interfere the assay of DIF by the described SW-AdAS

Table 4 Assay of DIF in its pharmaceutical formulations by the described SWAdAS voltammetric and the reported spectrophotometric methods [15]

Sample	Claimed value	Described voltammetric method (% R ± SD)		Reported spectrophotometric method [15]
		Calibration curve	Standard addition	
Doloban®	250 mg DIF/tablet	98.72 ± 0.61 $F = 3.81$ $t = 0.72$	98.4 ± 0.85	99.15 ± 1.19
Dolozal®	500 mg DIF/tablet	99.34 ± 0.89 $F = 1.42$ $t = 2.08$	100.5 ± 0.79	100.63 ± 1.06
Maxipan®	200 mg DIF + 200 mg NAP/tablet	98.20 ± 1.38 $F = 1.63$ $t = 0.69$	100.5 ± 0.79	98.74 ± 1.08

Theoretical F-value = 6.39 and t-test = 2.3 at 95% confidence limit for $n_1 = n_2 = 5$

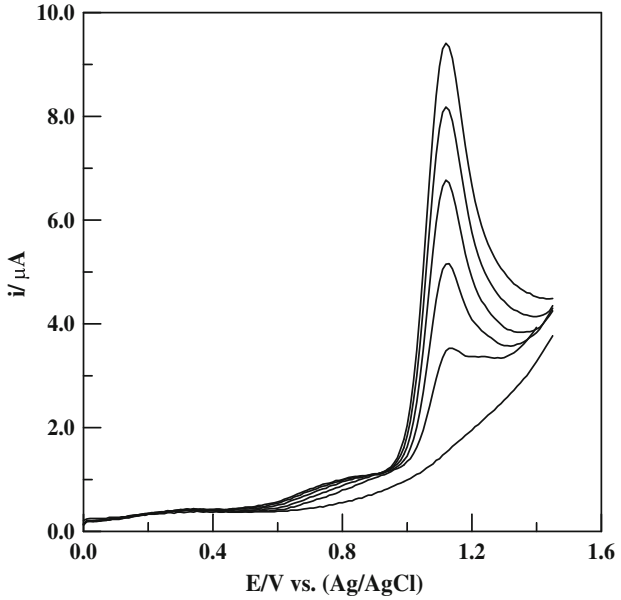


Fig. 7 SW-AdAS voltammograms for successive additions of DIF spiked in human serum sample, recorded in acetate buffer of pH 5.0 following preconcentration at 10% MMT-Ca-modified CPE by adsorptive accumulation at 0.0 V for 300 s. Each addition affected 1×10^{-8} M DIF. Other instrumental parameters are as those given in Fig. 5

voltammetric method. On the other side, voltammograms of human serum spiked with a binary mixture (DIF + NAP) showed no interference from NAP even at concentrations higher than those achieved therapeutically. The described SW-AdAS voltammetric method was then successfully applied for assay of DIF in spiked human serum without prior extraction, taking into consideration the medium exchange method described in the experimental section. Variation of the peak current versus concentration of DIF was linear within the range 1×10^{-8} to 2×10^{-7} M (Fig. 7) followed the regression equation; i_p (μA) = 162.51C (μM)–0.267 ($r = 0.996$ and $n = 10$). Mean percentage recovery of DIF in human serum was 99.16 ± 1.03% ($n = 5$). The achieved LOD was 3×10^{-9} M (0.75 ng mL^{-1}).

3.4.3 Pharmacokinetic studies

DIF is almost completely metabolized and its main metabolites are acyl glucuronide, phenolic glucuronide, and sulfate conjugate [3]. Since –OH group is the unique electroactive center in the drug molecule, these metabolites should be electro-inactive. Therefore, the described SW-AdAS voltammetric method might allow a selective determination of DIF in human blood without interferences from its metabolites. This advantage was proved by studying pharmacokinetics of DIF in plasma of two healthy

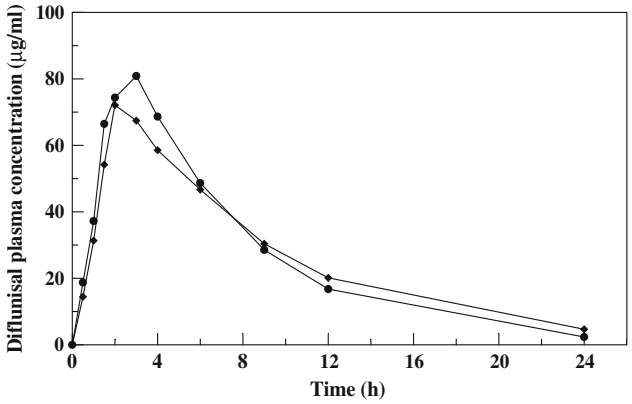


Fig. 8 Mean plasma concentration–time profiles for two male subjects following an oral administration of a single Dolozal® tablet, 500 mg DIF

Table 5 Pharmacokinetic parameters estimated for two male volunteers following an oral administration of a single Dolozal® tablet, 500 mg DIF

Parameter/unit	Estimated values*	
	Subject (1)	Subject (2)
C_{max} (μg mL^{-1})	80.87	72.11
t_{max} (h)	3.0	2.0
AUC_{0-24} (μg h mL^{-1})	647.78	646.07
$AUC_{0-\infty}$ (μg h mL^{-1})	678.30	717.46
K_{el} (h^{-1})	0.077	0.065
$t_{1/2}$ (h)	9.00	10.66

* Average of three measurements

male volunteers following the administration of a single oral Dolozal® tablet, 500 mg DIF. The obtained plasma concentration–time profiles of the two subjects are shown in Fig. 8. The following parameters were assessed for the period of 0–24 h: area under the plasma concentration–time curves from time zero to the last measurable sample time (AUC_{0-24}) and to infinity ($AUC_{0-\infty}$); maximum plasma concentration (C_{max}); time of the maximum concentration (t_{max}); elimination constant (K_{el}), and elimination half-life time ($t_{1/2}$), Table 5. These pharmacokinetic parameters obtained for the two volunteers were in good agreement with those reported (http://www.Drugs.com/pro/diflunisal.html), [44].

4 Conclusion

A simple, low cost and effective MMT-Ca-modified CPE was constructed for the trace determination of DIF due to its capability to significantly enhancing the stripping peak current of DIF. Therefore, a highly sensitive and selective

SW-AdAS voltammetric method was developed for the assay of DIF in different formulations and human blood. Its accuracy reproducibility, simplicity, and selectivity suggest its application in quality control analysis, clinical laboratories, and pharmacokinetic studies.

Acknowledgment The author express his gratitude to Ramadan Specialized Hospital's staff (Tanta City, Egypt), for the very kind care of the two volunteers and for providing the great facilities in collection and treatments of plasma samples required for the pharmacokinetic studies.

References

1. Tempero KT, Cirillo VJ, Steelman SL (1977) Br J Clin Pharmacol 4:315
2. Hannah J, Ruyle WV, Jones H, Matzuk AR, Kelly KW, Witzel BE, Holtz WJ, Houser RA, Shen TY, Sarett LH (1978) J Med Chem 21:1093
3. Verbeeck RK (1993) Conjugation reactions in man: studies with diflunisal. In: Jeffery EH (ed) Human drug metabolism. From molecular biology to man. CRC Press, Boca Raton, pp 81–88
4. Dickinson RG, King AR, McKinnon GE, Hooper WD, Eadie MJ, Herkes GK (1993) Br J Clin Pharmacol 35:609
5. Muralidharan B, Gopu G, Vedhi C, Manisankar P (2008) Appl Clay Sci 42:206
6. Manisankara P, Selvanathanb G, Vedhib C (2005) Appl Clay Sci 29:249
7. Manisankar P, Selvanathan G, Vedhi C (2006) Talanta 68:686
8. Navrátilová Z, Kula P (2000) Fresenius J Anal Chem 367:369
9. Kula P, Navrátilová Z (2001) Electroanalysis 13:795
10. Huang W, Yang C, Zhang S (2002) Anal Bioanal Chem 374:998
11. Ji Z, Hu S (2004) Collect Czech Chem Commun 69:1590
12. Sun D, Wan C, Li G, Wu K (2007) Microchim Acta 158:255
13. Abdel-Hay MH, Galal SM, Ragab MAA (2007) Taiwan Pharm J 59:157
14. Sayin F, Kir S (2004) Fabad J Pharm Sci 29:121
15. Trskova RA, Rychlovský PB, Nemcová IBC, Turek PB (1996) Pharmazie 51:550
16. Murillo Pulgarín JA, Alañón Molina A, Sánchez-Ferrer Robles I (2008) Anal Chim Acta 625:47
17. Maher HM (2008) J Fluorescence 18:909
18. Murillo Pulgarín JA, Alañón Molina A, Fernández López P, Sánchez-Ferrer Robles I (2007) Anal Chim Acta 583:55
19. Pérez-Ruiz T, Martínez Lozano C, Tomás V, Carpena J (1998) Fresenius J Anal Chem 361:492
20. Panadero S, Gómez-Hens A, Pérez-Bendito D (1996) Anal Chim Acta 329:135
21. Muñoz De La Peña A, Moreno MD, Durán-Merás I, Salinas F (1996) Talanta 43:1349
22. Lianidou ES, Ioannou PC, Polydorou CK, Efstathiou CE (1996) Anal Chim Acta 320:107
23. Maciá A, Borrull F, Calull M, Aguilar C (2007) Trends Anal Chem 26:133
24. Ding YAB, Garcia CDA (2006) Electroanalysis 18:2202
25. Vera-Candioti LA, Gil García MDB, Martínez Galera MB, Goicoechea HCA (2006) J Chromatogr A 1211:22
26. Kot-Wasik A, Débska J, Wasik A, Namieśnik J (2006) Chromatographia 64:13
27. Debska J, Kot-Wasik A, Namiesnik J (2005) J Sep Sci 28:2419
28. El Haj BM, Al Ainri AM, Hassan MH, Bin Khadem RK, Marzouq MS (1999) Forensic Sci Int 105:141
29. Lapicque F, Netter P, Bannwarth B, Trechot P, Gillet P, Lambert H, Royer RJ (1989) J Chromatogr 496:301
30. Loewen GR, MacDonald JI, Verbeeck RK (1989) J Pharm Sci 78:250
31. Streete PJ (1989) J Chromatogr 495:179
32. Ray JE, Day RO (1983) J Pharm Sci 72:1403
33. Midskov C (1983) J Chromatogr 278:439
34. Sayin F, Kir S (2001) J Pharm Biomed Anal 25:153
35. Wang J, Freiha BA (1983) Anal Chim Acta 148:79
36. Zuman P (1969) The elucidation of organic electrode processes. Academic Press Inc., New York, 21 pp
37. Zornoza A, Sánchez M, Vélaz I, Fernández L (1999) Biomed Chromatogr 13:111
38. Gosser DK (1994) Cyclic voltammetry. VCH, New York
39. Bard A, Faulkner LR (2001) Electrochemical methods: fundamentals and applications, 2nd edn. Wiley, New York, 236 pp
40. Swartz M, Krull IS (1997) Analytical method development and validation. Marcel Dekker, NY, 62 pp
41. Brooks MA (1996) In: Kissinger PT, Heineman WR (eds) Laboratory techniques in electroanalytical chemistry, 2nd edn. Marcel Dekker, New York
42. Christian GD (1994) Analytical chemistry, 5th edn. Wiley, London, 36 pp
43. Ewing GW (1995) Instrumental methods of chemical analysis, 5th edn. Lippincocott-Raven, Philadelphia, PA, p 464
44. Macdonald JI, Wallace SM, Mahachai V, Verbeeck RK (1992) Eur J Clin Pharmacol 42:471