Highly sensitive voltammetric and impedimetric sensor based on an ionic liquid/cobalt hexacyanoferrate nanoparticle modified multi-walled carbon nanotubes electrode for diclofenac analysis

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ABSTRACT
This paper describes the development of 1-methyl-3-butylimidazolium chloride ionic liquid/cobalt hexacyanoferrate nanoparticle modified multi-walled carbon nanotubes nanocomposite paste electrode for the electrocatalytic and adsorptive stripping voltammetric and impedimetric determination of diclofenac (DIC) in real samples. The nanocomposite was prepared by a simple chemical method and was characterised by scanning electron microscopy, Fourier transform infrared spectroscopy and atomic absorption spectroscopy. Also, the electrochemical behaviours of the modified electrode and the electrocatalytic oxidation of DIC were investigated in detail by cyclic voltammetry, chronoamperometry and electrochemical impedance spectroscopy techniques. The kinetic parameters such as electron transfer coefficient, and apparent rate constant for the redox reaction between DIC and the modified electrode were also determined using electrochemical approaches. It was found that the modified electrode exhibited excellent electrocatalytic activity toward the oxidation of DIC, and under the optimised conditions, the linear response range and detection limit were found to be 1.0–100.0 and 0.3 μM, respectively using the differential pulse voltammetry method. The proposed method was applied for the sensitive and selective determination of diclofenac in the urine samples and pharmaceutical formulations with satisfactory results.

KEYWORDS
carbon nanotube; cobalt hexacyanoferrate nanoparticle; diclofenac; ionic liquid; electrocatalysis

1. Introduction
Diclofenac (DIC) is a well-known representative of non-steroidal anti-inflammatory drugs (NSAIDs) with strong anti-pyretic, analgesic and anti-inflammatory properties. Anti-inflammatory and analgesic properties of NSAIDs are widely exploited in the management of musculoskeletal diseases. These drugs are applied both for acute and long-term chronic cases [1]. DIC is used to relieve the symptoms of many diseases such as rheumatoid arthritis, osteoarthritis, non-articular rheumatism and sport injuries. Patients
are frequently given special formulations of diclofenac or a co-treatment agent as a therapeutic strategy to attenuate the gastrointestinal tract complications that limit the use of diclofenac and other NSAIDs [2–5]. Therefore, the development of sensitive and rapid method capable of determining drug amount in pharmaceutical and clinical monitoring is important. Up to now, different means and techniques have been employed for the determination of diclofenac sodium, such as capillary electrophoresis [6], high performance liquid chromatography [7–10], spectrophotometry [1,11,12], spectrofluorometry [13–15], gas chromatography [16], potentiometry [17,18] and some voltammetric methods [19–25]. To date, electroanalytical techniques have been used for the determination of a wide range of drug compounds with the advantages that there is, in most instances, no need for derivatisation and that these techniques are less sensitive to matrix effects than other analytical techniques. Additionally, application of electrochemistry includes the determination of electrode mechanism. Redox properties of drugs can give insights into their metabolic fate or their in vivo redox processes or pharmacological activity.

The unique electrical properties, high chemical stability and high surface-to-volume ratio of carbon nanotubes (CNTs) have been intensively researched for electrocatalytic and sensing applications.[26–29]. Composite materials based on integration of CNTs and some other materials to possess properties of the individual components with a synergistic effect have gained growing interest.[30–32]. Among the various reagents used for electrode modification, transition metal hexacyanoferrates, a class of polynuclear inorganic compounds, have attracted great attention because they belong to a group of excellent electron transfer mediators [33–36]. Modification of an electrode surface with these compounds can be achieved by many different methods, including entrapping them in biopolymers [37] and electrodeposition [38]. Among the various metal hexacyanoferrates, cobalt hexacyanoferrate (CoHCF) has been studied intensively due to its well-defined and reproducible electrochemical responses, because both the oxidised and reduced CoHCF structures seem to be fairly open and permit transport of alkali-metal cations providing charge balance during redox reactions. CoHCF films can be easily fabricated on various surfaces [37], which make it an obvious candidate for electroanalytical applications such as electron transfer mediator, electrochromic devices and ion selective devices. Up to now, the modified electrodes based on CoHCF have shown good electrocatalytic activity toward a variety of biologically important substrates, such as thiols [34], hydrazine [35], bovine insulin [36], ascorbic acid [39], dopamine epinephrine, and norepinephrine, [40], paracetamol [41], tryptophan [42], and also for fabrication of biosensors [37].

The objective of the current work is to develop a simple and sensitive method for the determination of diclofenac (DIC), based on interesting synergetic effects of CoHCF nanoparticle decorated on multi-walled carbon nanotubes (MWCNTs) electrocatalyst. The mentioned nanocomposite is prepared as paste working electrode in the presence of 1-Butyl-3-methylimidazolium chloride (BMIMCl) room temperature ionic liquid (RTIL), named as MWCNTs/CoHCF/IL. RTILs can possess archetypical properties such as high intrinsic conductivity, high thermal stability, low volatility, high polarity, high viscosity and wide electrochemical windows [43]. On the other hands, ionic liquid (IL) is a suitable binder for preparation of carbon paste electrodes or modification of other solid state electrodes such as glassy carbon [44–46]. Here, we describe a cyclic voltammetry (CV), differential pulse voltammetry (DPV), chronoamperometry and electrochemical impedance spectroscopy (EIS) studies of DIC at a mentioned MWCNTs/CoHCF/IL electrode, in comparison to the
unmodified MWCNTs paste electrode using non-conductive paraffin as the binder. Also, the direct electrochemical behaviour of diclofenac on this new electrode was carefully investigated and further applied to the detection in real samples with satisfactory results.

2. Experimental

2.1. Materials

The MWCNTs were bought from Iran’s Research Institute of Petroleum Industry and synthesised by chemical vapour deposition with a diameter of 8–15 nm, a length 50 μm and the purity of 98%. Also, potassium hexacyanoferrate, cobalt(II) chloride hexahydrate, potassium chloride were analytical grade and used without purification. BMIMCl with purity ≥98.0% purchased from Sigma-Aldrich. Also, high viscosity paraffin oil (density = 0.88 g cm⁻³) from Fluka was used for preparation of CNT paste electrode, and diclofenac was purchased from Fluka. All aqueous solutions were made with demineralised water, which was further purified with a Milli-Q system (Millipore) with the resistivity not less than 18.0 MΩ at 25°C. Universal buffer (boric acid, phosphoric acid, acetic acid and sodium hydroxide, 0.1 M) solutions with different pH values were used for the electrochemical studies.

2.2. Apparatus

Electrochemical measurements were carried out in a conventional three-electrode cell, powered by an electrochemical system comprising the Autolab system with PGSTAT 12 and FRA2 boards (Eco Chemie B.V., Utrecht, The Netherlands). The system was run on a PC using general purpose electrochemical system (GPES) and frequency response analysis (FRA) 4.9 software. For impedance measurements, a frequency range of 100.0–10.0 Hz was employed. The AC voltage amplitude used was 5 mV, and the equilibrium time was 10 min. The MWCNTs/CoHCF/IL paste electrode, a graphite electrode and a saturated Ag/AgCl reference electrode were employed as a working, auxiliary and reference electrode, respectively. The MWCNTs/CoHCF/IL nanocomposites were characterised by scanning electron microscopy (SEM) with a Philips XL Model 30 microscope. Fourier transform infrared (FT-IR) spectra were recorded on JASCO/680 Plus spectrophotometer in transmission mode at a resolution of 4 cm⁻¹. Atomic absorption spectrometer was used for the determination of Co atom contents of MWCNTs/CoHCF/IL nanocomposite using a Perkin-elmer/2380 instrument.

2.3. Preparation of the MWCNTs/CoHCF/IL paste electrode

The MWCNTs, similar to other reports [29], were first purified and carboxylated by refluxing the nanotubes in the 2.0 M HNO₃ for 10 h, and washed until the pH of the solution was neutral, then dried at room temperature. 0.5 g of carboxylated CNTs was dispersed and homogenised in 50.0 ml acetonitrile solvent under ultrasonic agitation, then it was diluted to 250.0 ml by adding 25 ml of a solution containing 0.05 M hexacyanoferrate and 0.05 M KCl, and demineralised water. The final nanotube suspension was further homogenised under ultrasonication for 10.0 min. CoHCF nanoparticles were synthesised and deposited on the nanotubes by dropwise addition of 50.0 ml of a 0.01 M aqueous solution
of CoCl₂ to a 250 ml of the above MWCNTs suspension. After complete addition, the liq-
uid was vigorously agitated for 5 min, then immediately subjected to filtration. A 0.4 μm
Millipore cellulose filter membrane was used in the filtration. The product was continuously
washed with deionised water, then collected and dried overnight at room temperature.

MWCNTs paste electrode was prepared by hand-mixing of 0.90 g of purified CNT powder
and 0.10 g paraffin, and mixed well for 20 min until a uniformly wetted paste was
obtained. The paste was then packed into a glass tube (geometric surface area 0.0434 cm²).
Electrical contact was made by pushing a copper wire down the glass tube into the back of
the mixture. When necessary, a new surface was obtained by pushing an excess of the paste
out of the tube and polishing it on a weighing paper. MWCNTs/CoHCF/IL paste electrode
was prepared by mixing of 0.3 g of BMIMCl ionic liquid, 0.7 g of the liquid paraffin and
0.90 g of MWCNTs–CoHCF nanocomposite powder. Then the mixture was mixed
well for near to 50 min until a uniformly wetted paste was obtained. A portion of the paste
was filled firmly into one glass tube as described above to prepare the related working

2.4. Preparation and analysis of real samples

Contents of 10 diclofenac tablets (Darou Pakhsh Pharmaceutical Company), labelled with
amount of 25.0 mg per tablet, were completely homogenised. 15.0 mg of powders was
accurately weighted and dissolved under ultrasonication in 100.0 ml of water. 4.0 ml of
the resultant solution was diluted 10 times in 0.1 M pH 7.0 buffer containing 0.2 M NaCl
electrolyte, and it was used for the electrochemical analysis.

The fresh human urine samples were collected in dark glass containers, filtered through
0.22 μm pore size membrane, stored in refrigerator and analysed within 8 h after collec-
tion. Without any pre-treatment, samples were diluted with ultrapure water to the working
range of the determination of DIC, then it was used for analysis. The standard
addition method was used for the determination of analyte in real samples.

The cyclic voltammograms were recorded by cycling the potential between +0.1 and
+1.0 V (versus Ag/AgCl) at a scan rate of 50 mV s⁻¹. Differential pulse voltammetric anal-
ysis of real samples were performed in the universal buffer solution with pH 7.0, by scan-
ning the potential between 0.3 and 0.8 V at 10 mV s⁻¹ and a 50 mV pulse amplitude. The
content of diclofenac in the samples was determined by the standard addition method.

3. Results and discussion

3.1. Characterisation and electrochemical behaviour of MWCNTs/CoHCF/IL
electrode

The method of CoHCF nanoparticles deposition on the CNTs is relatively simple. By just
mixing cobalt ions and MWCNTs suspension containing hexacyanoferrate ions at room
temperature, nanoparticle growth takes place. After mixing and vigorously agitating, the
synthesis process produced dark brown aqueous suspensions. The suspension was subjected
to filtration immediately after synthesis. Excess of hexacyanoferrate solution removed as
permeate was initially yellow, then turned to clear. Atomic absorption spectrometry studies
showed that the amount of cobalt in the final nanocomposite is near to 0.2%. 
The dispersing state and homogeneity of the carboxylated MWCNTs and their nanocomposites with CoHCF were examined by SEM. Figure 1 shows a typical SEM image that confirms the formation of CoHCF nanoparticles, displaying particles or some agglomerates with the typical spherical shape having the diameter of around 10.0–200.0 nm, decorated on the CNTs.

FT-IR technique was used to further characterise the presence of CoHCF nanoparticles in the MWCNTs modified with CoHCF nanoparticles (see Figure 2). Here, the FT-IR spectra of functionalised MWCNTs and $K_4Fe(CN)_6$ were recorded and compared to MWCNTs/CoHCF nanocomposite. In the FT-IR spectrum of nanotubes (Figure 2(a)), the peaks at 1720 and 1575 cm$^{-1}$ correspond to $\nu$(C=O, $-\text{COOH}$) and $\nu$(C=O, $-\text{COO}^-$), respectively [47], indicating the presence of $-\text{COOH}$ (carboxylic acid) and $-\text{COO}^-$ groups on the surface of CNTs. The obvious peak at about 3450 cm$^{-1}$ is attributed to the presence of hydroxyl groups. The spectrum of $K_4Fe(CN)_6$ (Figure 2(b)) is split

![Figure 1. Typical SEM images of CoHCF-MWCNTs nanocomposite.](image1.png)

![Figure 2. The FT-IR spectra of the carboxylated MWCNTs (a), $K_4Fe(CN)_6$ (b) and MWCNTs/CoHCF/IL nanocomposite (c).](image2.png)
into three components at 2074, 2085 and 2097 cm
1 owing to the stretching vibration \( \nu(C=\text{N}) \) which is a common feature for hexacyanoferrates [48]. The FT-IR spectrum of MWCNTs/CoHCF nanocomposite (Figure 2(c)), which was recorded in a transmission mode, exhibits a relatively strong peak at 2107 cm
1 which is the peak characteristic of the stretching of the –CN group in the \( \text{Fe}^{\text{III}}-\text{CN}-\text{Co}^{\text{II}} \) links [49]. So the oxidation state of the cobalt and iron in CoHCF nanotubes can be expressed as \( \text{Fe}^{\text{III}}-\text{CN}-\text{Co}^{\text{II}} \). This peak slightly shifted to the higher wave numbers that could be explained by the \( \pi-\pi \) stacking interaction between MWCNTs and CoHCF.

CV was also employed to investigate the electrochemical properties of MWCNTs/CoHCF/IL paste electrode in the various buffered aqueous solutions, from pH 2.0 to 10.0. A typical cyclic voltammogram (see Figure 3) in pH 7.0 and the potential scan rate 50 mV s
1 exhibits two anodic and corresponding cathodic peaks with \( E_{\text{pa}} = 0.49/E_{\text{pc}} = 0.42 \) V and \( E_{\text{pa}} = 0.85/E_{\text{pc}} = 0.72 \) V vs. saturated Ag/AgCl reference electrode. According to previous reports [39], the redox reactions with the involvement of \( \text{K}^+ \) can be expressed as follows:

\[
\begin{align*}
\text{Co}^{\text{II}}_{1,5} [\text{Fe}^{\text{III}}(\text{CN})_6] + e^- + \text{K}^+ & \rightarrow \text{KCo}^{\text{II}}_{1,5} [\text{Fe}^{\text{II}}(\text{CN})_6] \quad (1) \\
\text{KCo}^{\text{II}} [\text{Fe}^{\text{III}}(\text{CN})_6] + e^- + \text{K}^+ & \rightarrow \text{K}_2\text{Co}^{\text{II}} [\text{Fe}^{\text{II}}(\text{CN})_6] \quad (2)
\end{align*}
\]

The experimental results show well-defined and reproducible anodic and cathodic peaks related to \( \text{KCo}^{\text{II}}_{1,5} [\text{Fe}^{\text{II}}(\text{CN})_6]/\text{Co}^{\text{II}}_{1,5} [\text{Fe}^{\text{III}}(\text{CN})_6] \) redox couple with quasi-reversible behaviour [37,38]. The peak separation potential, \( \Delta E_p = (E_{\text{pa}} - E_{\text{pc}}) \), is slightly greater than that 59/n mV expected for a reversible system. Figure 3 shows the comparison of voltammograms in the pure buffer solution and in the presence of 0.2 M NaCl and 0.2 M KCl electrolyte at the scan rate of 50 mV s
1. The figure demonstrates that the position of the redox peaks is dependent on the nature of the cation of the supporting electrolyte. In NaCl medium, the peak couple of the voltammogram in the presence of NaCl electrolyte is about 150 mV more negative than in KCl medium. This behaviour indicates that the electrolyte cations can slightly affect on the oxidation potential, which is to maintain the

Figure 3. Cyclic voltammograms of a MWCNTs/CoHCF/IL electrode in universal buffer solution (pH 7.0) containing 0.2 M NaCl (a), 0.2 M KCl (b) and without additive (c–e) at the potential scan rate of 50 mV s
1.
The electroneutrality of the modified electrode [50]. The film is doped with alkali-metal ions to provide charge neutrality within the film, and a net diffusion of ions in and out of the film takes place when the redox state of the film is changed.

The insertion of a counter cation into the film during reduction and its exclusion upon oxidation was verified by Kulesza et al. [51] using the electrochemical quartz crystal microbalance method. The formation of KCo$_{1.5}$[Fe$^{II}$(CN)$_6$], which is less strongly paired with potassium cations, is responsible for the appearance of a set of peaks at less positive potentials. An explanation of the dependence of the system’s behaviour on alkali-metal cations can be attempted by considering the size of a hydrated counter cation. CoHCF film has a face-centred cubic lattice crystal structure, Co(II) and Fe(II) or Fe(III), bridged by a $-$CN group, appearing alternatively at the sites of small cubes [13]. Owing to the rigidity of the crystal lattice of the CoHCF film, the diffusion of cobalt ions in it is very difficult. Only counter ions, such as K$^+$, are transported through the CoHCF film during the electrochemical redox process in order to maintain electroneutrality. It is likely that alkali-metal counter cations do not retain a complete hydration shell upon entrance to the crystal lattice because the cavity radius for CoCHF is 0.18 nm [52], the diameters for hydrated cations of Na$^+$ and K$^+$ are 0.36 and 0.24 nm, respectively. The counter cations must undergo a partial dehydration so that they found the appropriate size to enter the crystal lattice. The CoHCF film possesses greater selectivity for the K$^+$ ion as a counter ion [39].

The effect of varying the scan rate on the performance of the electrode was also studied. By increasing the scan rate, the CV peak currents of the CNT-CoHCF modified electrode increased in the range of 10.0–100.0 mV s$^{-1}$, and $\Delta E_p$ was slightly dependent on the scan rate, indicating that all of CoHCF mediators were efficiently connected on the MWCNTs surfaces. The cathodic and anodic peak currents increased linearly with the increase of potential scan rate, and the following equation obtained, respectively: $I(\mu A) = -0.7921 \times \nu (V\ s^{-1}) + 1.0215$, $R^2 = 0.9928$ and $I(\mu A) = 0.9576 \times \nu (V\ s^{-1}) - 2.0301$, $R^2 = 0.9967$, suggesting that the electrochemical reaction of MWCNTs-CoHCF modified electrode is an adsorption-controlled process.

The surface concentration of the electroactive mediator known as electrochemical rugosity or surface coverage $\Gamma$ is given by the expression [53]:

$$\Gamma = \frac{Q}{nFA}$$

where $Q$ is the integrated charge under the oxidation peak in CV for the CoHCF modified electrode, calculated from the area under the peak, $n$ is the number of electrons consumed in the unit electrode reaction ($n = 1$ in the present case) and the other symbols have their usual meaning. The surface coverage $\Gamma$ for the modified electrode calculated at different scan rates from 10.0 to 100.0 mV s$^{-1}$. The results show that $\Gamma$ value decreases as the scan rate increases from $3.0209 \times 10^8$ to $2.1421 \times 10^8$ mol cm$^{-2}$, respectively. Thus, the electron transfer process becomes more reversible at lower potential scan rates.

### 3.2. Electrocatalytic oxidation of diclofenac on the modified electrode

Figure 4 shows the typical cyclic voltammograms of 10.0 µM DIC in a pH 7.0 buffer solution on bare MWCNTs and MWCNTs/CoHCF/IL paste electrodes between 0.1 and +0.7 V at the scan rate 50.0 mV s$^{-1}$. This figure illustrates the cyclic voltammetric
responses of a bare CNT paste electrode in the presence of DIC (curve a) and modified electrode (curves b and c) in the absence and presence of DIC analyte, respectively. At the surface of the unmodified electrode, the direct oxidation of DIC starts from 420.0 mV and produces an anodic peak at the potential of 630.0 mV, with a depressed cathodic peak near to 230.0 mV. Under the identical conditions, the MWCNTs/CoHCF/IL paste electrode gives increased peak currents for DIC and the oxidation peak reduce near to 100.0 mV.

Kinetic studies by chronoamperometry confirm the electron transfer rate in the presence and absence of 10.0 μM DIC. At the intermediate times, the catalytic current ($I_C$) is dominated by the electrocatalytic oxidation rate of diclofenac. So the rate constant for the reaction between DIC and MWCNTs/CoHCF/IL is determined according to the method described in the literature [54],

$$\frac{I_C}{I_L} = \gamma^{1/2} \frac{\pi^{1/2}}{2} \text{erf}(\gamma^{1/2}) + \exp(-\gamma) / \gamma^{1/2}$$  \hspace{1cm} (2)

where $I_C$ and $I_L$ show current in the presence and absence of DIC, respectively. $\gamma = kC_ot$ ($k$ is the catalytic rate constant (M$^{-1}$ s$^{-1}$), $t$ the time elapsed (s) and $C_o$ is the concentration of diclofenac) is the argument of error function. Where $\gamma$ exceeds 2, the error function is almost equal to 1 and the above equation can be considered as follows:

$$\frac{I_C}{I_L} = \gamma^{1/2} \frac{\pi^{1/2}}{2} = \pi^{1/2} (kC.ot)^{1/2}$$  \hspace{1cm} (3)

According to Figure 5, the slopes of the $I_C/I_L$ vs. $t^{1/2}$ for 10.0 μM DIC on MWCNTs/CoHCF/IL was determined and $k$ was calculated to be 73.3 M$^{-1}$ s$^{-1}$. The rate constant for
10.0 μM DIC on MWCNTs paste electrode was calculated near to 21.8 M⁻¹ S⁻¹. Therefore, by using CoHCF as an electron mediator and RTILs in the matrix of the modified electrode, the overpotential for the anodic oxidation of diclofenac becomes considerably lower and the rate of the heterogeneous electron transfer is increased. This suggests an efficient electrocatalytic reaction of DIC on the MWCNTs/CoHCF/IL paste electrode.

In this study, we used the above process on the MWCNTs/CoHCF/IL paste electrode by adsorptive stripping voltammetry in the open circuit potential for the determination of diclofenac in the urine samples and pharmaceutical formulations and optimised the various parameters that can affect on the amount of that electrocatalytic oxidation signal.

### 3.3. Effect of pH

The peak currents for the oxidation of diclofenac (I(DIC)) on the modified electrode closely depend on the pH of buffer solution, but the current of CoHCF (I(CoHCF)) is rather constant in the pHs lower than 8.0. Experimental results in the pure buffer solutions and in the presence of 10.0 μM DIC at different pHs from 2.0 to 10.0 have been shown in Figure 6. It can be observed that the oxidation peak current of diclofenac rises as increasing pH, and it declines after attaining a maximum near to pH 7.0. Also, the potential peaks (E_p) are relatively constant in the pHs from 2.0 to 8.0, indicating that hydrogen ions in this oxidation reaction do not participate. For the pHs > 8.0, a minor hydrolysis of CoHCF nanoparticles can lead to shifting the potential peaks and the reduction of the corresponding currents (I(CoHCF)). In overall, the electrocatalytic oxidation of DIC on the surface of modified electrode is more favoured under pH 7.0 condition than in highly acidic or basic mediums. This appears as a gradual growth in the anodic peak current and a simultaneous decrease in the cathodic peak current of cyclic voltammograms. Therefore, pH 7.0 was chosen as the optimum pH for electrocatalysis.
3.4. Effect of potential scan rate

The effect of the potential scan rate on the electrocatalytic property of MWCNTs/CoHCF/IL paste electrode toward DIC oxidation was studied by CV in the presence of 10.0 μM analyte at pH 7.0 buffer solution (not shown). The results showed that with an increase in the scan rate, the peak potential for the electro-oxidation of diclofenac shifts to more positive potentials, suggesting a kinetic limitation in the reaction between the redox sites of CoHCF and DIC. In addition, the cathodic current would increase with increasing scan rate, because in short time-scale experiments, there is no enough time for catalytic reaction to take place completely. However, the oxidation current of DIC increased linearly with the square root of the scan rate of potentials, and the following equation obtained:

\[ I (\mu A) = 1.5790 \times \nu^{1/2} (V s^{-1}) - 2.7292, \quad R^2 = 0.9955, \]

suggesting that at sufficient overpotentials the process is diffusion controlled. In order to get the information on the rate determining step, the values of \( \alpha n_a \) (where \( \alpha \) is the transfer coefficient and \( n_a \) is the number of electrons involved in the rate determining step) were calculated for the oxidation of DIC at pH 7.0 at both modified and unmodified MWCNTs according to the following equation [54]:

\[ \alpha n_a = 0.048/(E_p - E_{p/2}) \]  

where \( E_{p/2} \) is the potential corresponding to \( I_{p/2} \). The values for \( \alpha n_a \) were found to be 0.52 and 0.32 for the oxidation of DIC at the surface of the MWCNTs/CoHCF/IL and unmodified MWCNTs paste electrode, respectively. These values clearly show that not only the overpotential for DIC oxidation is reduced at the surface of MWCNTs/CoHCF/IL, but also the rate of the electron transfer process is greatly enhanced, this phenomenon is thus confirmed by the larger \( I_{pa} \) values recorded during CV on the modified electrode.

The electrocatalytic process that is compatible with the observed behaviour is presented in Scheme 1. During the anodic scan, the ferrocyanide present in the mediator is electro-chemically oxidised to ferricyanide, which in turn chemically oxidises the DIC present in the solution to the radical species \((\text{Ph})_2 = N \) [55] and getting itself reduced to ferrocyanide. This process can be repeated a number of times, resulting in electrocatalytic oxidation of diclofenac by the mediator present in the modified electrode.
3.5. EIS studies

EIS was also employed to investigate the oxidation of diclofenac on both bare MWCNTs and MWCNTs/CoHCF/IL paste electrodes. Figure 7 shows the Nyquist plots of the impedance (Ω cm²) for the mentioned electrodes recorded at 0.45 V as dc-offset for some concentrations of DIC in pH 7.0. The equivalent circuit compatible with the Nyquist diagram recorded in the absence and presence of DIC is depicted in the inset of this figure. In this circuit, Rₛ, CPE and Rₘ represent solution resistance, a constant phase element corresponding to the double-layer capacitance, and the charge transfer resistance associated with the oxidation of DIC species. W is a finite-length Warburg short-circuit term coupled to Rₘ, which accounts for the Nernstian diffusion. In the presence of DIC, the diameter of the semicircle decreases, confirming the electrocatalytic capability of the mentioned electrocatalyst for the oxidation of diclofenac. This is due to the instant chemical reaction of DIC with the high-valence CoHCF species. The catalytic reaction for the oxidation of DIC that occurred via the participation of MWCNTs and CoHCF electrocatalysts (KCoII₁₅[FeII(CN)₆]) virtually caused an increase in the surface concentration of low valence species of CoHCF (CoII₁₅[FeIII(CN)₆]), and the charge transfer resistance declined, depending on the concentration of DIC in the solution (see Table 1). This behaviour is consistent with the result of CV and chronoamperometry (see Figures 4 and 5). Impedance of CPE and W elements can be expressed as [56,57]

\[ Z_{\text{CPE}} = Y_0^{-1} (j\omega)^{-n} \]  \hspace{1cm} (5)

\[ Z_{W} = Y_0^{-1} (j\omega)^{-1/2} \]  \hspace{1cm} (6)

where \( Y_0 \) (the admittance parameter, S cm⁻² s⁻ⁿ) and \( n \) (dimensionless exponent) are two parameters independent of frequency; \( j = (-1)^{1/2} \) and \( \omega \) = angular frequency = \( 2\pi f \). \( Z_{\text{CPE}} \) corresponds to the constant phase element (CPE) impedance. \( Y_0 = C_d \) only when \( n = 1 \), and \( n \) is related to \( \alpha \) (phase angle) by \( \alpha = (1 - n) 90^\circ \). So, \( n = 1 \) and \( \alpha = 0 \) stand for a perfect capacitor, and lower \( n \) values directly reflect the roughness of the electrode surface. When \( n = 0.5 \), it is equal to a Warburg impedance. When \( n = 0 \), CPE is reduced to a resistor.

In the mentioned circuits, the charge transfer resistance of the electrode reaction is the only circuit element that has a simple physical meaning describing how fast the rate of charge transfer during electro-oxidation of diclofenac changes with the electrode potential.

\[ 2 \text{KCoII}_{1.5}[\text{FeII}(\text{CN})_6] \xrightarrow{\text{Electrode Reaction}} 2 \text{CoII}_{1.5}[\text{FeIII}(\text{CN})_6] + 2e^- + 2\text{K}^+ \]

\[ 2 \text{CoII}_{1.5}[\text{FeIII}(\text{CN})_6] + \begin{array}{c} \text{COOH} \\ \text{Cl} \end{array} \text{NH} \text{Cl} \xrightarrow{\text{Chemical Reaction}} \begin{array}{c} \text{COOH} \\ \text{Cl} \end{array} \text{N} \cdot \text{Cl} \xrightarrow{\text{+ 2KCoII}_{1.5}[\text{FeII}(\text{CN})_6]} \]

Scheme 1. Proposed electrocatalytic process for the oxidation of diclofenac on the MWCNTs/CoHCF/IL paste electrode.
or analyte concentration. The most widely accepted explanation for the presence of constant phase elements and the appearance of depressed semicircles in the Nyquist plots is the microscopic roughness, causing an inhomogeneous distribution in the solution resistance as well as in the double-layer capacitance.

**Table 1.** The values of the elements in equivalent circuit and the corresponding relative errors for the oxidation of DIC on the MWCNTs and MWCNTs/CoHCF/IL paste electrodes.

| Electrode               | C_{DIC} (μM) | R_{ct} (Ω cm^2) | Y_0 \times 10^{-4} (S cm^{-2} s^5) | \rho \times 10^4 (Ω cm^2) | \sigma |
|------------------------|--------------|-----------------|---------------------------------|----------------------|-------|
| MWCNTs                 | 0.0          | 6945.6 (3.1%)   | 0.1010 (3.7%)                  | 0.8266 (2.8%)        | –     |
| MWCNTs                 | 10.0         | 3550.7 (3.6%)   | 0.1631 (1.9%)                  | 0.8123 (3.3%)        | –     |
| MWCNTs/CoHCF/IL        | 0.0          | 1236.8 (3.8%)   | 0.1523 (3.1%)                  | 0.7432 (2.6%)        | 0.1813 (2.6%) |
| MWCNTs/CoHCF/IL        | 10.0         | 958.6 (2.2%)    | 0.1981 (2.9%)                  | 0.8641 (2.1%)        | 0.2644 (3.4%) |
| MWCNTs/CoHCF/IL        | 50.0         | 639.4 (2.5%)    | 0.1289 (1.7%)                  | 0.8316 (1.4%)        | 0.3833 (1.2%) |
| MWCNTs/CoHCF/IL        | 100.0        | 442.3 (3.7%)    | 0.1338 (3.2%)                  | 0.8122 (2.2%)        | 0.5223 (2.3%) |
| MWCNTs/CoHCF/IL        | 150.0        | 315.8 (3.6%)    | 0.1355 (3.7%)                  | 0.8221 (2.7%)        | 0.5823 (3.9%) |

**Figure 7.** The Nyquist diagrams of the impedance ($Z_{IL}$ vs. $Z_{ct}$) on the (A) bare MWCNs in the absence (a) and presence of 10.0 μM (b), and (B) MWCNTs/CoHCF/IL paste electrodes in the presence of 10.0 μM (a), 50.0 μM (b), 100.0 μM (c) and 150.0 μM (d) DIC by applying a bias of 0.45 V and ac voltage with 5 mV amplitude in a frequency range from 10.0 Hz to 100.0 kHz in pH 7.0. Insets show the equivalent circuits compatible with the Nyquist diagrams.
Table 1 shows some values of the equivalent circuit elements obtained by fitting the experimental results. The goodness of the fit can be judged by the estimated relative errors presented in the parentheses. According to the values of the electrical equivalent elements reported in this table, upon increasing the concentration of DIC on the MWCNTs/CoHCF/IL electrode, the charge transfer resistances \(R_{ct}\) decreased due to the facile occurrence of the faradic process related to the electro-oxidation process. The electron transfer resistance, \(R_{ct}\), for the 10.0 \(\mu\)M DIC on the unmodified nanotube, and MWCNTs/CoHCF/IL electrode equals to 3550.0 and 958.6 \(\Omega \text{ cm}^2\), respectively, indicating the very faster charge transfer rate for oxidation of diclofenac on the modified electrode surface, due to the electrocatalytic effect of nanocomposite on the electro-oxidation process. The apparent electron transfer rate constant \(k_{app}\) can be obtained from the following conventional equation [54]:

\[
k_{app} = \frac{RT}{n^2F^2AR_{ct}C}
\]

where \(n\) is the number of electron transferred \((n = 1)\), \(A\) is the microscopic area of the electrodes, \(C\) is the concentration of diclofenac \((\text{in mol cm}^{-3})\), \(R\), \(T\) and \(F\) have their usual meanings. Thus, the value of \(k_{app}\) on the MWCNTs/CoHCF/IL is about 3.7 times higher than that of the unmodified electrode, demonstrating enhancement of charge transfer reaction kinetics.

3.6. Performance of the system for the determination of diclofenac

Because DPV has a much higher current sensitivity than CV, it was used for the determination of diclofenac in real samples. The calibration plot of DIC determination is linear in the concentration region of 1.0–100.0 \(\mu\)M with detection limit of 0.3 \(\mu\)M (see Figure 8). Using DPV, the optimum conditions were selected as pH 7.0 (universal buffer) by scanning the potential between 0.3 and 0.8 V at 10 mV s\(^{-1}\) and a 50 mV pulse amplitude. Equation of linear least square calibration curves over this ranges is \(I(\mu A) = 0.2075 \times C_{DIC} + 1.4517\) \((R^2 = 0.9907)\).

To prove the precision and practicability of the proposed method, the reproducibility and storage stability of the sensor were examined. The relative standard deviation (RSD) of the modified electrode response to 10.0 \(\mu\)M DIC was 3.5\% \((n = 8)\). The RSD of the responses to diclofenac for five different electrodes prepared in the same conditions was 4.6\%. Also, the modified electrode showed constant response without much change in peak potential and peak current when it was cycled continuously for 2 h at a scan rate of 50 mV s\(^{-1}\). These results showed that such kinds of electrodes exhibited good reproducibility. Furthermore, the long-term stability of the MWCNTs/CoHCF/IL was tested over a three-week period. The CV of DIC at the surface of modified electrode after storage in the ambient conditions showed that the oxidation peak potential of DIC was unchanged and the anodic peak current was only decreased less than 3.0\% of the initial oxidation peak current.

3.7. Interference studies

Under optimised experimental conditions described earlier, the effects of some foreign species on the determination of DIC at 10.0 \(\mu\)M level were evaluated in detail.
The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error less than 5.0% for determination of DIC. Five hundred-fold of Na$^+$, K$^+$, Ca$^{2+}$, Mg$^{2+}$, NH$_4^+$, stearate, urea, SO$_4^{2-}$ and NO$_3^-$; 180-fold of Cl$^-$ and Br$^-$; fivefold of glucose, sucrose, ascorbic acid, uric acid and dopamine, have almost no influence on the current response of DIC. All these indicate that the amount of diclofenac peak current is not affected by various conventional cations, anions and organic substances, and usually can exist in urine samples.

### 3.8. Application

To evaluate the applicability of proposed method, the recovery of DIC was determined in the urine and tablet samples by adding the standard value of DIC to them. The standard addition method was used for the analysis of prepared samples. The data given in Table 2 show the satisfactory results for analytical determination of diclofenac in the real samples.

### Table 2. Determination of DIC in pharmaceutical formulations and urine samples by DPV technique in pH 7.0 buffer solution.

| No. | Sample | Added (µM) | Found$^a$ (µM) | Recovery (%) | RSD (%) |
|-----|--------|------------|----------------|--------------|---------|
| 1   | Urine  | 100        | 98.0 ± 0.1     | 98.0         | 0.3     |
| 2   |        | 500        | 480.8 ± 0.5    | 96.1         | 1.8     |
| 3   |        | 1000       | 1020.4 ± 1.0   | 102.0        | 2.6     |
| 4   | Tablet | 100        | 101.6 ± 1.7    | 101.6        | 2.6     |
| 5   |        | 200        | 196.5 ± 2.9    | 98.25        | 0.8     |
| 6   |        | 300        | 294.6 ± 3.8    | 98.2         | 0.4     |

$^a$Average of five replicate measurements.
4. Conclusion

In conclusion, a relatively homogenous mixture of CoHCF nanoparticles, ionic liquid and MWCNTs nanocomposite was easily prepared by a simple chemical method, and it was employed for the fabrication of a sensitive diclofenac electrochemical sensor in the urine samples and pharmaceutical formulations. The synergetic effects between the nanocomposite components and adsorptive stripping in the open circuit potential enhance the electrocatalytic action of CoHCF for the oxidation of DIC, resulting in a sensitive and selective voltammetric sensor for this drug with wide linear detection range and acceptable reproducibility. Kinetic studies by chronoamperometry and EIS confirm that the electron transfer rate of modified electrode is near to 3.4–3.7 times greater than bare MWCNTs paste electrode. Also, the values for $\alpha_{n,a}$ were found to be 0.52 and 0.32 for the oxidation of DIC at the surface of the MWCNTs/CoHCF/IL and bare MWCNTs paste electrodes, respectively. This nanocomposite has a potential to be greatly used for designing a variety of sensors and biosensors.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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