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Electrochemical Methodology for NSAID's Determination and its Interaction with Steroid Dexamethasone

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The new generations of Non-Steroidal Anti-inflammatory Drugs (NSAIDS) are COX-2 selective cyclooxygenase-2 inhibitors (analgesic and anti-inflammatory) capable to cause adverse gastrointestinal events due to drug interactions between one or more co-administered medicines. This causes alteration of the efficacy or toxicity of the co-administered drug. This study presents an electrochemical method (CV) evaluation to study the interactions of Naproxen Sodium and Piroxicam with Dexamethasone (Steroid) under optimized conditions. The proposed method is electrochemically diffusion controlled as both drugs showed different diffusion coefficients (Piroxicam: 2.188×10^{-7}; Naproxen Sodium: 3.755×10^{-5}). The method has good reproducibility and validated according to ICH guide lines (R^2 = 0.9994 for Naproxen Sodium and 0.9991 for Piroxicam). The interactions were confirmed by the FTIR studies indicated the variation of wave number and intensity of significant peaks thereby revealed possible interaction sites.

Keywords: Cyclic voltammetry, FTIR, NSAIDs, Piroxicam, Naproxen Sodium, Dexamethasone.

1. INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are frequently used worldwide at an annual 11.9% increasing rate. These drugs are prescribed mainly for the orthopedic conditions such as fractures, osteoarthritis and soft-tissue injuries. To support the clinical trial, patient samples are regularly analyzed to measure the drug in complex media and bio-fluids therefore validated and reliable analytical methods are highly desirable [1]. Different conditions like cancer, inflammatory bowel diseases, arthritis mental disorder and increase in the cardiovascular risk are related with inflammation. Several investigations have reported the higher risk of cardio-vascular diseases by the NSAIDs [2]. The NSAIDs possess

inhibition capacity of cyclo-oxygenase enzyme [3-5] and anti-tumor activity by decreasing the size and number of carcinogen-induced problems [6].

In the present work we have focused on two NSAIDs Piroxicam and Naproxen Sodium. The Piroxicam provides a very effective treatment of musculoskeletal disorder, rheumatoid arthritis, osteo-arthiritis and sports injuries. Side effects associated with Piroxicam are gastrointestinal effects such as bleeding ulcers [7]. Naproxen is a well-known NSAID, mostly used in improper condition of joints and acts by inhibiting cyclooxygenase enzyme resulting in decreased biosynthesis of specific prostaglandins [8]. It is important to know that when these NSAIDs are allowed to interact with steroidal drugs, the risk of peptic ulcer increases. For example when taken alone, the side effects of indomethacin causes gastric lesion. The severity of these lesion increases remarkably if prednisolone is also prescribed at the same time. Excessive doses of nimuliside do not cause any gastric lesion unless concomitant with prednisolone [9]. Dexamethasone is a widely used steroidal (glucocorticoid) drug. Because of anti-inflammatory, immuno-suppressive properties it has been used for the treatment of asthma, rheumatoid arthritis and helps to suppress immunological reactions in patients [10]. For the analysis of Dexamethasone several methods have been reported inside biological fluid and also in pharmaceutical formulations like High Performance Liquid Chromatography [11], HPLC-ion spray mass spectrometry [12], Micellar electro-kinetic capillary chromatography [13], Highly sensitive liquid chromatography-tandem mass spectrometry [14], solid-phase extraction and liquid chromatography [15], reverse phase HPLC [16], HPLC tandem mass spectrometry [17-18], solid phase extraction and a Monolithic Column [19-20]. There are several methods reported for the analysis of NSAIDs such as electro-reduction or oxidation of Piroxicam and Naproxen by the different authors [21-27]. The anodic oxidation of Naproxen sodium has been carried out on a surface of Pt electrode using linear sweep, cyclic and differential pulse voltammetry (DPV) [28]. A fast and novel electroanalytical method for analysis of Naproxen in pharmaceutical formulations was developed using Batch Injection Analysis (BIA) with pulsed Amperometric techniques [29]. Another electroanalytical method attempt was made by using boron-doped diamond (BDD) electrode [30]. Zinc oxide nanoparticles and multi walled carbon nanotubes (MWCNTs) modified electrode was also used as a sensitive and fast tool for the investigation of Naproxen [31].

In the current study we have developed a cyclic voltammetric method to monitor interactions between the two NSAIDs and Dexamethasone (steroid). To best of our knowledge this is first, detailed, low-cost and systematic electroanalytical study (method) for the effective monitoring of drug-drug interactions. The method indicated that these drugs should not be taken together because their interactions may alter the bioavailability, bioactivity, gastrointestinal absorption and dissolution of second drug [32].

2. EXPERIMENTAL

2.1 Reagents and chemicals

Merck Pharmaceutical provided Naproxen Sodium standard and Nabi-Qasim Pharmaceutical supplied standard Piroxicam. Pharmaceutical formulation of Naproxen and Piroxicam were from Synflex (550 mg of Martin Dow Limited) and Feldene 10 mg of Pfizer Pakistan Limited. Tablets of

Dexamethasone (0.5 mg, Dexatex Syntax Pharma) were used for all the experiments. Buffer solution di-sodium hydrogen phosphate/potassium dihydrogen phosphate (PBS) of pH 7.0 was purchased from Merck KGaA, 64271 Darmstadt Germany. For polishing of electrode alumina powder (0.2 µm, CH Instrument) was used. All solutions were prepared in PBS (pH 7.00). The solvent DMSO and salt KBr (For FTIR) were purchased from Merck. The $NaClO_4$ and $LiClO_4$ were purchased from Sigma Aldrich.

2.2 Procedure

A three electrode system was used to perform all the experiments on electrochemical analyzer (CHI760D, Austin, USA). The saturated calomel electrode (SCE) was used as the reference electrode (RE). Glassy Carbon (GC), Gold (Au) and Platinum (Pt) electrodes having area 0.0766 cm^2, 0.02065 cm^2 and 0.02065 cm^2 respectively were used as the working electrode and Pt wire as a counter electrode. All the cyclic voltammogram were recorded at 25 ^oC. Prior to each run, the working electrode was cleaned with alumina powder (0.2 µm). FT-IR spectroscopy was performed in FTS-65 Bio-rad. The proposed method is validated according to International Council for Harmonisation (ICH) guidelines (QSEM: Quality, Safety, Efficacy, Multidisciplinary), an organization to fulfill the requirements of Pharmaceuticals for human use (*www.ich.org/home.html*).

3. RESULTS AND DISCUSSION

We have evaluated the percent availability of a commonly prescribed steroidal drug Dexamethasone in the presence of two well-known NSAIDs Naproxen Sodium and Piroxicam by using the cyclic voltammetry supported by the FTIR. Prior to the discussion of invitro interactions of the NSAIDs with Dexamethasone we would like to elaborate about the method development and validation in the following discussion.

3.1 Method development

To develop an adequate method for the analysis of Naproxen Sodium and Piroxicam parameters like the working electrode, supporting electrolyte, solvent and scan-rate were checked to get well-defined peaks by using the cyclic voltammetry.

3.1.1 Choice of solvent and supporting electrolytes

Different solvents and supporting electrolytes were tested (drugs completely soluble) to get the optimized conditions for the development of a method.

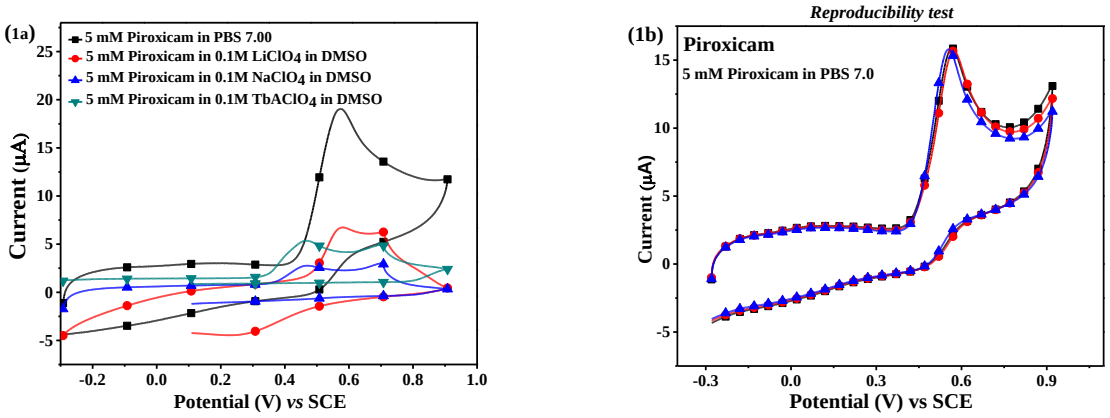

Figure 1. Choice of solvent and supporting electrolyte for Piroxicam at ν = 50 mV/s @ GCE (WE), Pt wire (CE) and SCE as the reference electrode (a); Reproducibility test (b).

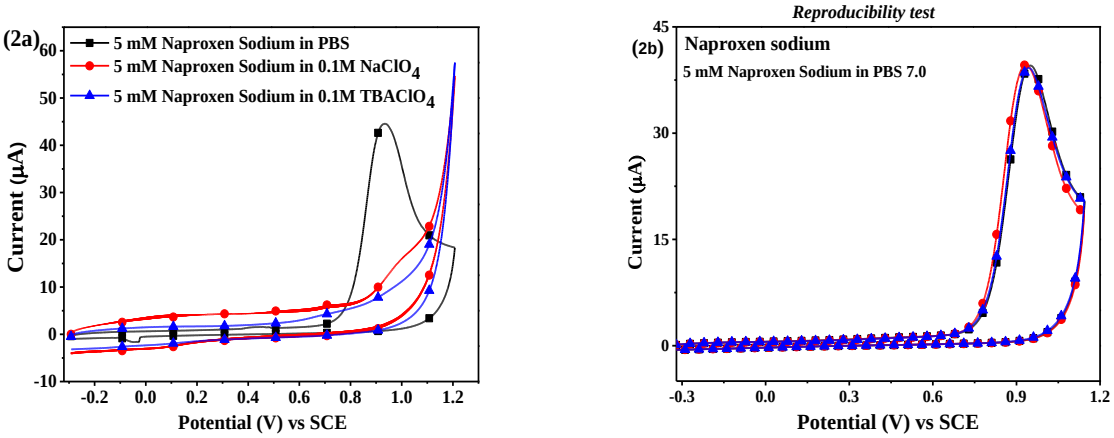

Figure 2. Choice of solvent and supporting electrolyte for 5 mM Naproxen Sodium at ν = 50 mV/s @ GCE (WE), Pt wire (CE) electrode and SCE (RE); Reproducibility test (b).

Fig. 1a and Fig. 2a show the voltammograms for the selection of supporting electrolyte and solvent for Naproxen Sodium and Piroxicam respectively. We found that buffer solution di-sodium hydrogen phosphate/potassium dihydrogen phosphate (PBS) of pH 7.00 is an efficient system because it enabled us to observe a well-defined and reproducible peak (CV, Fig. 1b, 2b) close to the necessary human body physiological conditions during the analysis.

3.1.2 Choice of electrode

For the CV curves of Piroxicam we used the potential window -0.3 to 0.7V with three different working electrodes: Platinum (Pt), Gold (Au) and Glassy carbon electrode (GC). Only one anodic peak was observed at potential values 566 mV, 580 mV and 560 mV for GC, Au and Pt respectively. On the reverse scan no cathodic-peak was observed, therefore Piroxicam showed an irreversible charge transfer reaction [33]. The comparison of current density from all three electrodes (Fig. 3, voltammograms)

indicates large differences. In addition, when GC was used as the working electrode, the peak currents were more reproducible. Therefore GC working electrode was selected as an appropriate electrode for further analysis of Piroxicam.

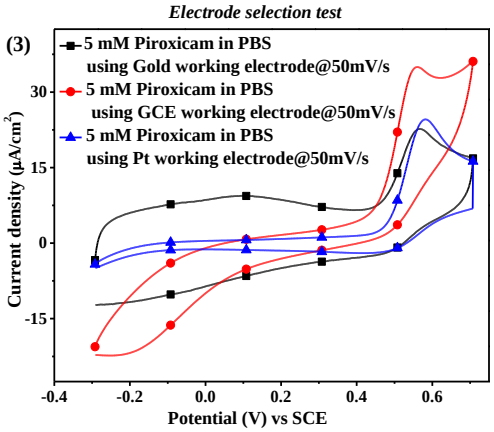

Figure 3. Electrode Selection for 5mM Piroxicam @ ν = 50 mV/s, Pt wire (CE), SCE (RE) using PBS buffer of pH 7 as supporting electrolyte.

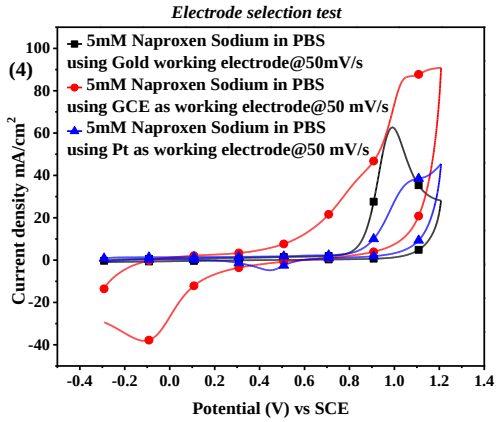

Figure 4. Electrode Selection: 5 mM Naproxen Sodium @ ν = 50 mV/s, Pt wire (CE), SCE (RE) using PBS buffer of pH 7 as supporting electrolyte.

For the Naproxen Sodium, we used electrode potential window -0.3-1.2 V to get an appropriate redox behavior (Fig. 4). The GC electrode showed irreversible charge transfer oxidation peak at 950 ± 15 mV with peak current 45.58 µA. Although the peak potential of Au and Pt were close but the GC (WE) showed best current density.

3.1.3 Effect of scan rate

Scan-rate defines whether a redox system is adsorption or diffusion controlled therefore CVs were recorded for the 5 mM solution (PBS buffer of pH 7) of Piroxicam (Fig. 5a) and Naproxen Sodium (Fig. 6a) at different scan rates by using the GC working electrode.

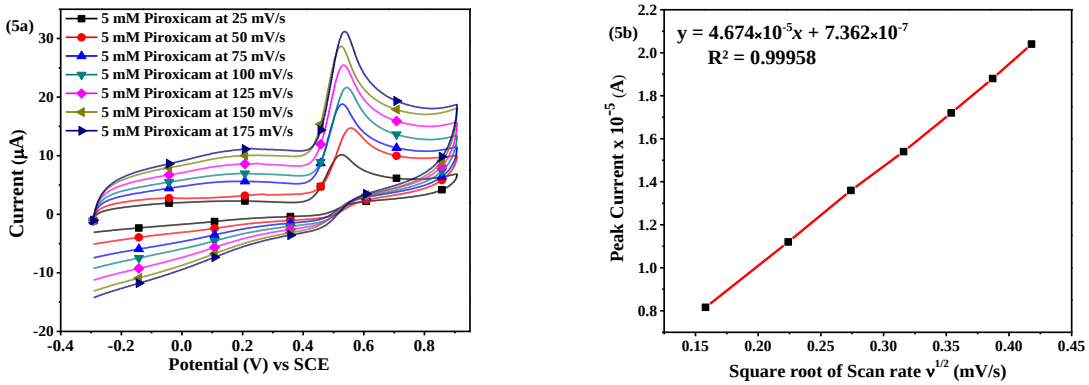

Figure 5. Overlay of CVs (a): 5mM Piroxicam @ different scan rates using PBS buffer (pH 7) as supporting electrolyte, Pt wire (CE), SCE (RE); linear relation between peak current (I_P) of Piroxicam *vs* $\nu^{1/2}$ (b).

The data obtained at different scan rates can be used to calculate the diffusion coefficients of both NSAIDs Piroxicam and Naproxen Sodium by using Randles-Sevcik equation. Diffusion coefficients values were calculated (eq. 1) from the slopes ($y = mx + c$, Fig. 5b, 6b) obtained by the plots between the peak current (I_p) *vs* square root of scan rate $\nu^{1/2}$ [34].

$$I_p = 2.99 \times 10^5 \, nAC(\alpha n D \nu)^{1/2} \quad (1)$$

Table 1. Diffusion coefficients of Piroxicam and Naproxen sodium @ GCE.

Drugs	*n*	Area of Electrode ($4\pi r^2$, cm^2)	α	Conc. of NSAIDs (mM/cm^3)	Slope *m*	Diffusion Coefficient $cm^2 . s^{-1}$
Piroxicam	1	0.07065	0.6	5×10^{-6}	4.674×10^{-5}	2.188×10^{-7}
Naproxen Sodium	1	0.07065	0.6	5×10^{-6}	2.594×10^{-4}	3.7554×10^{-5}

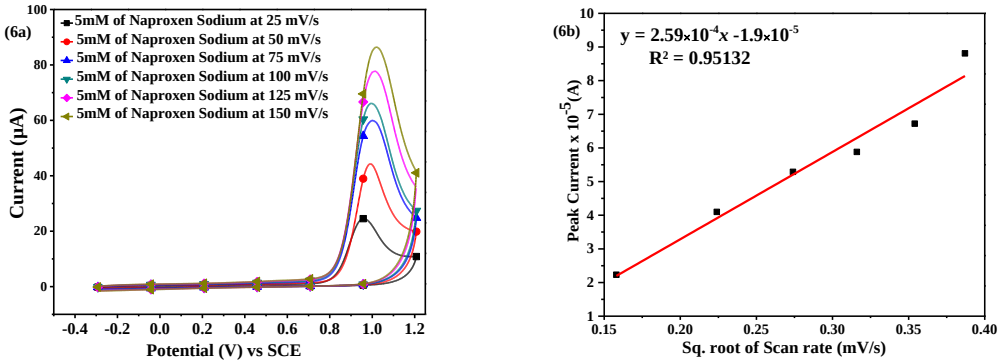

Figure 6. Overlay of CVs (a): 5 mM Naproxen Sodium at different scan rates using PBS buffer of pH 7.0 as supporting electrolyte @ Pt wire (CE), SCE (RE); linear relation between peak current (IP) of Naproxen Sodium *vs* $\nu^{1/2}$ (b).

Where A is the area of working electrode (cm^2), D is the diffusion coefficient (cm^2/s), 'C' concentration of the analyte (mM/cm^3), ν is the scan rate (V/s), *n* is the number of electrons transferred, *α* is the transfer coefficient, I_P is the peak potential current, from the above equation average values of diffusion coefficients were calculated (Table 1). The diffusion coefficients values indicated that the proposed method is diffusion controlled for both the NSAIDs.

3.2 Method validation

After the method development, the next step is method validation according to the ICH guide lines. This step helps to confirm the analysis method suitability.

3.2.1 Linearity of proposed method

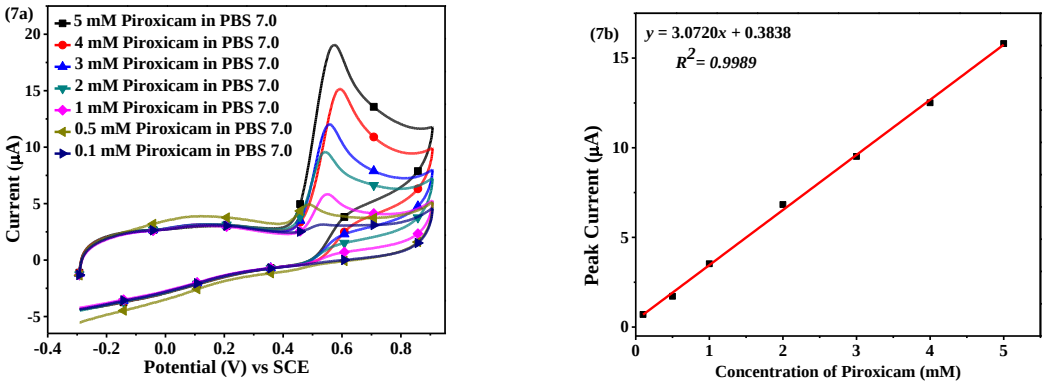

Figure 7. Overlay of CVs (a) at different concentrations of Piroxicam @ 50 mV/s (scan rate); GC (WE), Pt wire (CE) and SCE (RE) in PBS buffer 7.0; linear relation (Calibration Curve): concentration of Piroxicam *vs* Current response (b).

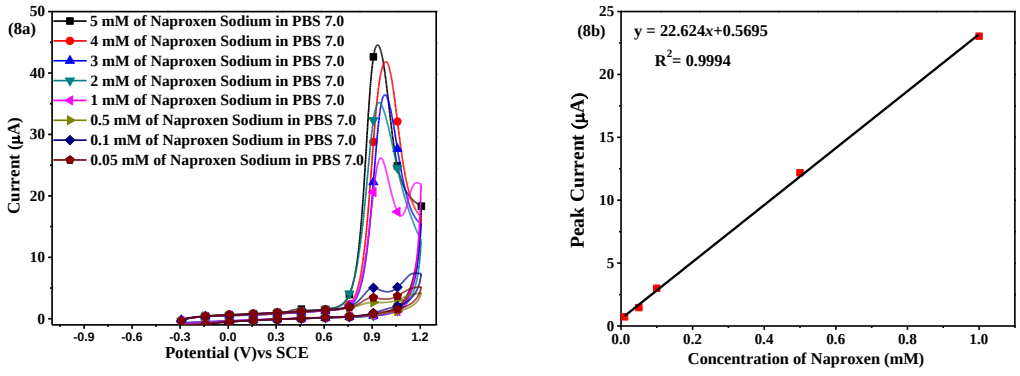

Figure 8. Overlay of CVs (a) at different concentration of Naproxen Sodiummonitored @ 50 mV/s (scan rate), GC (WE), Pt wire (CE) and SCE (RE) in PBS buffer 7.0 (a); linear relation (Calibration Curve): between different concentrations of Naproxen Sodium *vs* Current response (b).

The linearity of proposed method was determined by preparing a series of solutions with different concentration from 5-0.1 mM of Piroxicam standard. A very good linearity was achieved (R^2 = 0.9991, Table 2, Fig. 7b).

Table 2. Regression statistics and sensitivity of the proposed method.

Drugs	Regression Equation	Slope (*S*)	Correlation Coefficient (R^2)	LOQ (μM)	LOD (μM)
Piroxicam	$y = 3.072x + 0.3838$	3.072	0.9989	830.07	249.02
Naproxen	$y = 22.624x + 0.5695$	22.624	0.9994	103.34	31.00

To check the linearity of Naproxen Sodium by CV method, we prepared different standard concentrations of Naproxen Sodium (5-0.05 mM) and recorded their CVs (Fig. 8a). A very good linear relation was observed between the concentration (1 mM to 0.05 mM) and peak current (R^2 = 0.9994, Table 2, Fig. 8b).

3.2.2 Limit of detection (LOD) limit of quantification (LOQ)

The Limit of Detection (LOD) and Limit of Quantification (LOQ) for the CV method was calculated as LOD = 3.3 (standard deviation of blank/slope) and LOQ = 10 (standard deviation of blank/slope). The value of slope was determined from the calibration curves for the Piroxicam and Naproxen Sodium respectively (Table 2). For standard deviation of blank, PBS buffer (pH 7.0) was run five times and its current response was measured at same potential where analyte showed its peak potential at 950 mV for Naproxen Sodium and 566 mV for Piroxicam (Table 3). In Table 3 we calculate the standard deviation of five observations of both NSAIDs Piroxicam and Naproxen sodium; this is required for LOQ and LOD. For this we took least concentration of both analyte where analyte signal detected. Here is 0.1 mM for Piroxicam and 0.05 mM for Naproxen sodium.

Table 3. Standard deviation of blank for LOQ and LOD.

No.	Current (μA) PBS buffer pH 7.00 at 950 mV (Naproxen Sodium)	Current (μA) PBS buffer pH 7.00 at 566 mV (Piroxicam)
1	2.408	1.018
2	2.092	1.342
3	1.964	1.203
4	1.91	1.698
5	2.388	1.193
Mean	2.1524	1.2908
SD	±0.233847	±0.255013

3.2.3 Repeatability and reproducibility of proposed method

The precision of the proposed method was evaluated by its repeatability within a same day (Intraday) and reproducibility in three consecutive days (Interday). The repeatability and intermediate results of Piroxicam and Naproxen Sodium were reported as standard deviation and R.S.D. Intra-day precision of Piroxicam and Naproxen Sodium were calculated to be 0.0079 and 0.01241 respectively. Inter day precision of Naproxen Sodium and Piroxicam were 0.013 and 0.03868 respectively.

3.2.4 Robustness

To check the robustness of the proposed method, the pH of the system was varied (7.00±0.2). The CVs were recorded to check the effect of pH change on peak current and potential. The potential and current were not affected by the small pH changes.

3.2.5 Recovery studies

Table 4. Recovery studies of proposed method of Piroxicam and Naproxen Sodium.

Drugs		*Sample Concentration (mM)*	*Concentration of added Standard (mM)*	*% Recovery**	*Mean*
Piroxicam	120%	4	5	95.129	99.64
	100%	4	4	101.07	
	80%	4	3	102.73	
Naproxen	120%	2	3	101.13	99.08
	100%	2	2	99.782	
	80%	2	1	96.344	

Accuracy is the %analytes recovered by analyzing from a known added amount of standard into the sample solution. Nine samples of three different concentrations were evaluated. For this purpose 4 mM of sample of Piroxicam was added into 5 mM, 4 mM and 3 mM standard Piroxicam. The Table 4 shows %recovery of added standard drugs. Similar to above, nine samples of three different concentrations were evaluated. A 2 mM of sample of Naproxen Sodium was prepared and added into 3 mM, 2 mM and 1 mM standard Naproxen Sodium.

3.3 Interaction studies

After the successful development of cyclic voltammetric method, we employed it for the interaction studies. Patients taking steroids concomitantly with NSAIDs have more risk of upper gastrointestinal (GI) complications. Previous reports indicate that anti-inflammatory drugs treatment should be monotherapy and dose should be lowered if possible to decrease the chances of upper gastrointestinal complications. The steroid users who were concomitantly given NSAIDs showed

duration and dose dependent risk for developing a peptic ulcer disease, while the non-users of NSAIDs showed no risk for ulcer disease [35-36].

Table 5. Percent availability of two drugs at different time intervals.

No.	Time in min	Piroxicam and Dexamethasone		Naproxen Sodium and Dexamethasone	
		% Availability of Piroxicam	%Availability Dexamethasone	%Availability Naproxen Sodium	%Availability Dexamethasone
1	0	100	100	100	100
2	15	85.57	65.54	26.36	81.82
3	30	85.13	68.59	22.76	70.71
4	45	84.25	66.61	19.2	70.88
5	60	83.93	70.67	7.44	76.33

At first 5 mM Piroxicam and 5 mM Dexamethasone solutions were scanned (Fig. 9) by the CV (GC (WE) @ fixed scan-rate: 50 mV/s) in PBS buffer (pH = 7.0) then 5 mM solution of Piroxicam was mixed with 5 mM Dexamethasone for 15 min and the redox behavior was monitored. The individual Piroxicam showed peak potential at 560 mV and Dexamethasone at 470 mV but the solution containing equal amounts of both showed obvious decrease in the peak current. The observations infer that the two drugs interacted, confirmed by monitoring the peak current response and experimental %availability (Table 5). The discernment in %availability clearly indicates that there was lesser amount of drugs available for the redox reaction.

Similar to the above procedure 5 mM solution of Naproxen Sodium and 5 mM Dexamethasone in PBS 7.0 were placed in a cell with magnetic stirrer for 15 min and scanned (Fig. 10) by the CV. The same procedure was adopted to monitor the CV response (15 min interval, 1 h). The CV results showed that the formation of charge transfer complex resulted in the decrease of peak current. When the duration of interaction increases; the %availability of Naproxen Sodium and Dexamethasone drugs decreases. It is very likely that these drugs make a charge transfer complex due to donor (electron-rich) and acceptor (electron-deficient) nature of the two drugs. These observations suggest that co-administration of these two drugs can alter their effectiveness.

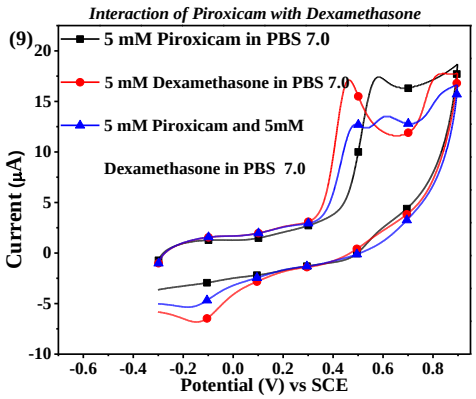

Figure 9. Interaction of Dexamethasone and Piroxicam monitored @ scan rate 50 mV/s; GC (WE), Pt wire (CE) and SCE (RE) in PBS buffer 7.0.

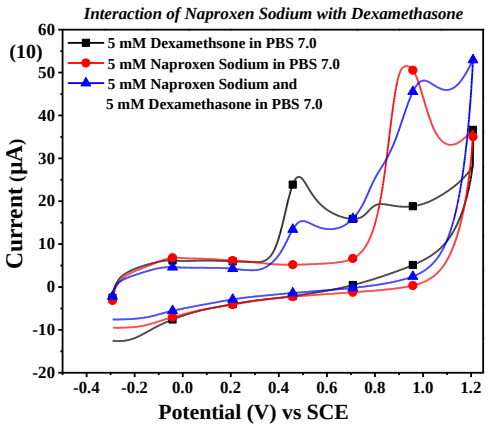

Figure 10. Interaction of Dexamethasone and Naproxen sodium monitored @ scan rate 50 mV/s; GC as WE, Pt wire as CE and SCE as RE in PBS buffer 7.00.

3.3.1 Interaction effect on diffusion coefficient

Diffusion coefficients were measured after the interaction of both drugs at certain intervals of time (Table 6). The table 6 shows that when the drugs started to interact; value of D_O decreased. Slow diffusion towards the surface of electrode is prognostic proof that there must be the formation of charge transfer complex in between two drugs which showed the hindrance for the molecules to move towards surface of electrode.

Table 6. Diffusion coefficients at different intervals of time (before and after interactions)

Time (min)	Interaction of Piroxicam and Dexamethasone		Interaction of Naproxen Sodium and Dexamethasone	
	Diffusion Coefficient Piroxicam D_O x 10^{-7} (cm^2/s)	Diffusion Coefficient Dexamethasone D_O x 10^{-7} (cm^2/s)	Diffusion Coefficient Naproxen Sodium D_O x 10^{-7} (cm^2/s)	Diffusion Coefficient Dexamethasone D_O x 10^{-7} (cm^2/s)
Before Interaction	4.48	3.53	3.013	3.53
15	3.28	1.51	2.003	2.366
30	3.24	1.66	1.848	2.149
45	3.78	1.56	1.315	1.980
60	3.06	1.62	1.937	2.660

3.3.2 Interaction effect on binding constant with respect to time

Cyclic voltammetry (CV) has been frequently used to find the binding strength of drugs when they interact with DNA. Since CV also runs backward scan therefore we can have some information

about the fate of interacting species. When a drug interacts with DNA the peak current and peak potential shows obvious changes. Therefore, the peak current and peak potential change can be used for the determination of binding related parameters [37].

Table 7. Binding constant of NSAIDs after interaction with Dexamethasone.

S. No.	Time (min)	Binding Constant *K* (l/mol) Piroxicam	Naproxen Sodium
1	15	33.697	558.647
2	30	34.915	678.69
3	45	37.387	840.721
4	60	38.279	2486.385

Binding constants were determined for piroxicam and naproxen sodium by the eq. (2)

$$\log\left(\frac{1}{[\text{Drug}]}\right) = \log K + \log\left(\frac{I}{I_0 - I}\right) \qquad (2)$$

Where *K* is the binding constant of drugs before the interaction of NSAIDs with Dexamethasone, I_0 is the initial current (absence of interaction) and *I* (presence of interaction) correspond to the current after the interaction of NSAIDs with Dexamethasone. The binding constants (Table 4) for Piroxicam and Naproxen Sodium with Dexamethasone are 33.697 mol^{-1} and 558.647 mol^{-1} respectively. With the passage of time the binding constants of both drugs increased which confirmed that the drug's binding (adduct formation) increased with time.

3.4 Spectroscopic investigation of interaction through FTIR spectroscopy

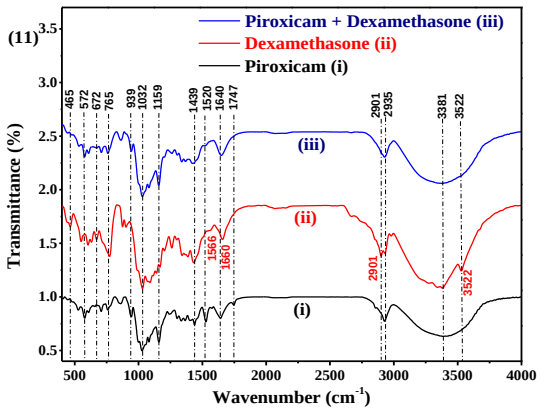

Figure 11. FTIR (KBr, cm^{-1}) spectra of Piroxicam, Dexamethasone and the Piroxicam-Dexamethasone adduct.

The FT-IR spectra (Fig. 11. 400 to 4000 cm^{-1}) of all three Piroxicam (Pir), Dexamethasone (Dex) and adduct were compared. The Piroxicam showed few sharp bands at variable wave numbers. At 3381 cm^{-1} a sharp absorption band is due to OH and NH stretching [38]. At 2928 cm^{-1} there is C-H stretching and at 1660 cm^{-1} Oxa acetic acid of Piroxicam was observed [39-40]. Other bands accredited to the following groups: 1566 cm^{-1} to the amide carbonyl (–CONH) stretching, 1520 cm^{-1} to the secondary amide stretching, 1439 cm^{-1} to the C-H and Ar-C=C- stretching, 1159 cm^{-1} to the S=O and a sharp absorption at 939 cm^{-1} corresponds to the SO_2-N. FTIR spectra of steroidal drug Dexamethasone showed bands at 3522 cm^{-1} (–OH free stretching) and 3381 cm^{-1} due to the -OH…H (hydrogen bonded) stretching [41]. The significant peak of Dexamethasone responsible for the C-H stretching appeared at 2935 cm^{-1} [42]. The main absorption band of Dexamethasone observed at 1260.7 cm^{-1} emerged due to C-F stretching while a peak at 1657.7 cm^{-1} is from the C=O stretching [43]. FTIR spectra of product of Piroxicam and Dexamethasone elucidated that Piroxicam undergoes interaction at –OH, –NH and –CONH functional groups [44]. The intensity of band absorption at 3381 cm^{-1} further confirmed that its interaction site is –OH and –NH. The disappearance of O-H stretching at 3522 cm^{-1} of Dexamethasone confirmed that O-H is the main site for interactions.

3.4.1 FTIR spectra of interaction between Naproxen sodium and dexamethasone

The FT-IR spectra (Fig. 12, 400 to 4000 cm^{-1}) of all three Naproxen Sodium, Dexamethasone (Dex) and adduct were compared. The FTIR spectra of pure Naproxen Sodium showed its distinctive peak at 1264 cm^{-1} due to the C–O stretching. At 1603 cm^{-1} a peak due to CO_2– stretching, a significant peak at 1630.9 cm^{-1} due to the C=C aromatic stretching and the presence of peak at 2935 cm^{-1} from aliphatic C–H stretch [45-46].

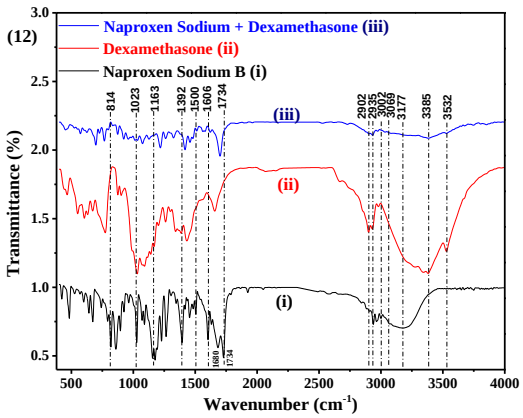

Figure 12. FTIR (KBr, cm^{-1}) spectra of Naproxen Sodium, Dexamethasone and Naproxen Sodium-Dexamethasone adduct.

The C-O stretching vibrations emerged at 1304 cm^{-1}, aromatic C=C stretching at 1630.9 cm^{-1} [47]. When Dexamethasone reacted with Naproxen Sodium considerable changes in their spectra can be attributed to their interactions confirming the most probable interaction sites. Intensity of several peaks (2935 cm^{-1}, 1734 cm^{-1} and 1163 cm^{-1}) decreased after interaction. Peak at 3522 cm^{-1} completely wiped

out which shows that O-H group of Dexamethasone is a binding site for the Naproxen Sodium. Absorption peak of Naproxen Sodium at 1680 cm^{-1} also diminished which indicated that $O=C-O^-$ is one of the interaction site of Naproxen Sodium and Dexamethasone.

4. CONCLUSIONS

We have described a GC working electrode based, diffusion controlled cyclic voltammetry (CV) method for the drug interaction studies of NSAIDs with Dexamethasone in PBS buffer (pH=7, solvent and supporting electrolyte) excellent due to similarity with human physiological conditions. The %availability of a commonly prescribed steroidal drug Dexamethasone in the presence of two well-known NSAIDs Naproxen Sodium and Piroxicam was explained by the CV. The interaction studies showed that the percent availability of both drugs decreased with the passage of time. FTIR spectra of individual drugs and adducts with Dexamethasone elucidated their interaction sites. Therefore we recommend that the two drugs should be taken separately.

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