# Introduction

## 1.1.Literature survey on nimesulide determination:

Nimesulide, methanesulfonamide, N-[4-nitro-2-phenoxyphenyl] is a new non-steroidal anti-inflammatory drug (NSAID), with analgesic and antipyretic properties [1,2] that dose not induce gastrointestinal ulceration [3]. It is an inhibitor of prostaglandin synthesis from arachidonic acid and of platelet aggregation [4,5].

For several years, pharmacological investigations of exogenous compounds or therapeutical agents have focused on a possible interaction with reactive oxygen species [6] in order to asses their capacity to prevent or minimize free radical damages to biological targets. As the species production that may aggravate the pathological manifestations of diseases [7], several commonly used or potential non-steroidal anti-inflammatory drugs (NSAIDs) have been tested as potential free radical scavengers.

The pharmacokinetic profile of nimesulide has been assessed in healthy volunteers after oral and rectal administration of the samples (tablet, sachet or suspension forms) [8]. The $pK_a$ of nimesulide was determined by potentiometric [9] and spectrophotometric [10] methods.

Nimesulide was determined by several methods, including liquid chromatography (LC) [11,12], high performance liquid chromatography (HPLC) in human plasma [13-16], and high-performance thin layer chromatography (HPTLC) [17]. The drug was also determined by spectrophotometric methods [18,19].

There have been few reports about the electrochemical determination of nimesulide. The development and validation of an adsorptive stripping voltammetry procedure for the drug has been described [20] applying the

optimized conditions, the linear range was 3.32 $x10^{-9}$ – 3.85 $x10^{-7}$ M and the dosage form mean recovery was 101.3 % with RDS of 1.3 % (n=9).

A flow system for the amperometric determination of nimesulide was described [21]. The referred system was made up of two parallel channels that shared the voltammetric detector of tubular configuration, whose movement in the manifold followed the concept of multi-site location of detector. In this way, after each measurement, the conditioning of the working electrode was possible through the passing by its surface of a generation solution without implying the alternation of the carrier that flowed in the analytical channel of the manifold. A series of recovery experiments were proceeded resulting in a mean value of 101.1 % with a R.S.D. of 0.7 %.

1.2.Literature survey on secnidazole determination:

Secnidazole, (hydroxyl-2-propyl)-1-methyl-2-nitro-5-imidazole), is an antimicrobic agent. This drug has pharmacological activity against intestinal and hepatic amebiasis, giardiasis and vaginal trichomoniasis [22].

Secnidazole was determined by HPLC in pharmaceutical formulations [23,24] and blood and urine [25]. It was also determined in human blood by gas chromatography [26] and in pharmaceutical dosage forms by supercritical fluid chromatography [27]. A spectrophotometric method was recently published about the determination of secnidazole in pharmaceutical formulation [28].

Few studies were published for the electrochemical behavior of nitroimidazole derivatives. The cyclic voltammetry of 4-nitoimidazole was performed in protic media, 0.1 M Britton-Robinson buffer containing 0.3 M KCl [29]. In alkaline solutions, the compound is reduced in one

electron reduction on the time scale of the cyclic voltammetric technique. The results obtained from this study in protic media differ substantially from other studies in aprotic media where in the nitro radical anion was not stable.

Cyclic voltammetry was used to examine the adsorptive behavior of secnidazole at (HMDE). A sensitive stripping voltammetric procedure for determination of the drug using differential pulse technique was applied [30]. The determination range, detection limit and precision of the method were determined. The method was applied to the analysis of secnidazole in urine.

1.3.Literature survey on nifuroxazide determination:

Nifuroxazide, [5-nitro-2-furaldehyde p-hydroxybenzoyl hydrazone] is a nitrofuran derivative widely used in human medicine for therapy of acute diarrhea from bacterial origin. This drug is mutagenic in bacteria and consequently it could has long term adverse effects on human health [31]. Nifuroxazide was determined by HPLC [32] in biological fluids. The properties of suspension formulations which contain nifuroxazide as active constituent were studied by spectrophotometry and polarography [33]. The polarographic determination depends on the reduction of the nitro-group, while spectrophotometric method depends on absorbance measurements at 373 nm.

The electrochemical properties of nifuroxazide have been investigated in aqueous and aqueous-DMF mixed solvents [34]. In aqueous media, a single, irreversible four-electron reduction occurs to give the hydroxylamine derivative. In mixed media, a reversible one-electron reduction to form a nitro radical anion takes place. Cyclic voltammetric studies show that the anion radical product is stable, although the nitro

radical anions intermediate shows a tendency to undergo further chemical reactions.

The voltammetric behavior of nifuroxime was investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) at bare and DNA-modified glassy carbon electrodes [35]. Based on the optimized voltammetric procedure, a sequential-injection stripping analysis (SISA) has been evaluated for the determination of the drug.

1.4.Literature survey on tinidazole determination:

Tinidazole, 1-[2-(ethylsulphonyl)ethyl]-2-methyl-5-nitro-1H-imidazole], is active against protozoa and anaerobic bacteria and used like metronidazole in range of infections [36]. Most of the methods reported for the analysis of tinidazole in biological fluids rely on the use of HPLC [37-39], gas chromatography (GC) [40], and packed column supercritical fluid chromatography [41].

The drug was determined by spectrophotometric methods in pharmaceutical formulations [42-44] and serum [45]. A UV-spectrophotometric technique was applied to the determination of the hydrolyzed drug [46]. A second-derivative spectrophotometric procedure has been developed for the simultaneous determination of tinidazole, furazolidone and diloxanide furoate in a commercial preparation [47].

The electrochemical behavior of tinidazole was studied by normal pulse and reverse pulse polarography [48]. In acidic aqueous solutions, the drug gives rise to two irreversible cathodic waves, while at higher pH, the limiting current of the first wave increases until only a single wave is observed. In strongly alkaline media, tinidazole decomposes; the kinetics of this process were studied by normal pulse and second-order voltammetry.

The electrochemical reduction of tinidazole has been carried out in aqueous solutions over a large pH range by differential-pulse (DP) polarography [49]. A method for the determination of tinidazole in Britton-Robinson buffer of pH 3.0, which allows quantification over the range 0.03-703 μg/ml, was proposed. The method was applied to the determination of the drug in tablets with mean recovery and relative standard deviation of 98.7 and 3%, respectively.

1.5.Literature survey on cinnarizine determination:

cinnarizine, [1-trans-cinnamyl-4-diphenylmethylpiperazine], is a piperazine derivative with histamine $H_1$-receptor and calcium channel blocking activity. It is used for the symptomatic treatment of nausea and vertigo caused by Meniers disease and other vestibular disorders. It is also used for the prevention and treatment of motion sickness [50].

Two different HPLC methods were applied to the determination of cinnarizine in plasma [51] and tablets [52]. A gas chromatographic method was reported for the cinnarizine determination in biological samples [53]. The drug was also determined by liquid chromatography (LC) and thin-layer chromatography in serum and pharmaceuticals [54].

Spectrophotometric methods were applied to determination of cinnarizine in dosage forms [55,56] and in a binary mixture containing antihistamines [57]. A method for flow-injection determination using surfactant-enhanced permanganate chemiluminesence was reported [58].

Direct potentiometric determination of cinnarizine has been applied using a potentiometric membrane sensors for the selective determination of the drug [59]. This method was applied to pharmaceutical formulations.

1.6.Literature survey on chlorodiazepoxide determination:

cholorodiazepoxide, is [7-chloro-2-(methylamino)-5-phenyl-3H-1,4-benzo diazepine-4-oxide]. The drug may inhibit monosynaptic and polysynaptic reflexes by acting as inhibitory neuronal transmitters or by blocking excitatory synaptic transmission. The drug may also directly depress motor nerve and muscle function [60, 61] and anxiety-related conditions including spastic colon [62].

The polarographic behavior of chlordiazepoxide and its reduction mechanism at a mercury electrode were studied in different media [63-67]. Chlordiazepoxide was determined in different samples using several electrochemical techniques, e.g. dc-polarography [68-71], differential-pulse polarography [72,73], differential-pulse stripping voltammetry [74] and square-wave polarography and voltammetry [75].

It was also determined spectrophotometrically [76-80], and after reduction by zinc or cadmium in a continuous system followed by atomic-absorption spectrometric detection [81].

Basic principles of DC polarography technique:-

Polarography is concerned with the study of voltage-current-time relationships during electrolysis carried out in a cell where one electrode is of constant potential (reference electrode) and the other has a very small surface area (working electrode). The technique commonly involves studying the influence of voltage changes on the current flowing in the cell. In dc polarography, the micro-electrode is usually a dropping mercury electrode (DME).

The current measured in polarography is the diffusion current resulting from the reduction of electro-active material. The factors affecting this current are given by Ilkovic equation [82] :

$$I_d = 607\, n\, D^{1/2}\, C\, m^{2/3}\, t^{1/6} \text{ ---------- } (1.1)$$

Where :

$I_d$ : the average diffusion current in microamperes during the life of the drop.

n : the number of electrons consumed in the reduction of one mole of the electro-active species.

D : the diffusion coefficient of the reducible or oxidizable substance expressed as $cm^2 s^{-1}$ .

C : its concentration in m mole $L^{-1}$ .

m : the rate of flow of mercury from the dropping electrode expressed in mg $s^{-1}$ .

t : drop time in seconds .

Basic principles of cyclic voltammetry technique:-

Cyclic voltammetry is usually performed with a small stationary electrode (solid electrode, or hanging mercury drop electrode ) immersed in an unstirred solution of reactant in a suitable solvent and electrolyte . The potential of the electrode is varied linearly with time in either the positive or negative direction until a switching potential is reached, at this direction of potential sweep is reversed and the potential returned to the original value. The current is measured throughout the experiment and the resulting i-E curve is called a voltammogram. The characteristic peaks in the voltammograms are caused by the formation of a depletion layer in solution near the electrode. The position of the peaks on the potential axis is related in a simple manner to the formal potential of the redox process. From the peak height and shape the reactant concentration and number of electrons could be determined. The sweep rate used in conventional experiments ranges from a few $mVs^{-1}$ up to a few hundred $Vs^{-1}$. Highly sweep rates introduce considerable experimental difficulties (e.g. double layer charging and iR drop effects can both very large) which generally restrict their applications. The method of recording the

voltammogram depends on the sweep rate; up to about 0.5 $Vs^{-1}$. An X-Y recorder is most convenient, but above this rate, the slow rate of the recorder will be insufficient to record the voltammogram without distorting it. In these cases a fast transient recorder, an oscilloscope and a microcomputer are required .

Cyclic voltammetry has been employed using stationary electrode in studying of mechanisms of electrode processes. It has been found to be practically useful in investigations of mechanisms of oxidation and reduction of a number of organic substances using either solid [83-87] or mercury electrodes [88-92]. In contrast to polarography, the substance produced during cathodic reduction remains in the neighborhood of the electrode surface and can be re-oxidized when the direction of polarization is reversed. From the intensity of the recorded oxidation or reduction currents and from the potentials at which the currents are observed, information on reversibility and irreversibility of the system and on chemical reactions accompanying electrode processes can be obtained.

Basic principles of stripping technique:-

Stripping analysis is an analytical technique that utilizes a bulk electrolyte step to preconcentrate the analyte from the sample solution at the surface of working electrode. In this case, the preconcentration step can be viewed as an effective electrochemical extraction in which the analyte is preconcentrated at the surface of electrode to a much higher level than it exists in solution. This preconcentration step is followed by an electrochemical measurements of the concentrated analyte.

The combination of a preconcentration step with advanced measurement procedures generates the extremely favorable singal-to- background ratio that characterizes stripping analysis therefore, a basic knowledge about voltammetry is necessary for a good understanding of the two main

voltammetric techniques of stripping analysis which are: cathodic stripping voltammetry (CSV) and anodic stripping voltammetry (ASV).

Stripping analysis on microelectrode:-

Stripping analysis has the ability to measure very low concentrations by an expensive instrument. Stripping analysis offers the advantage of species characterization. Comparative studies with other analytical techniques have emphasized the various advantages of stripping analysis[93,94]. The main limitation of stripping analysis is its restriction to about 30 metals. In addition, various nonmetals, such as halides, thiols or sulfides can be determined in the cathodic version of stripping analysis.

The ions or species accumulate at the surface of electrode at positive potentials then they are reduced on application of cathodic voltages scan . The sensitivity of cathodic stripping voltammetry depends on the amount that can be plated at a given period.

Different factors affecting the stripping technique are :

***1-The deposition step:***

The deposition step is usually carried out employing a controlled potential electrolysis for a definite time and under reproducible hydrodynamic (mass transport) conditions in the solution at the surface of electrode. The deposition potential imposed on the working electrode is chosen according to the species to be determined and is maintained for a deposition period depending on their concentration. However, the parameters governing this step are :

***(i) Deposition potential and time:***

The deposition potential, $E_d$, is applied to the working electrode to cause the compound of interest to deposit at its surface, this potential can be calculated from the Nernest equation. In practice, it is normally to check experimentally the effect of the deposition potential on peak current of interest and to use the value that yields the choice of deposition potential can provide some selectivity in the measurement. This value is sometimes helpful in resolution

of overlapping peaks. A non zero intercept may be observed in the $i_p$ versus $t_d$ plot because of additional compound plating during the scan from the deposition potential to the peak potential. However, fast scan rates, such as in case of linear scan voltammetry (LSV) and square-wave voltammetry (SWV), are useful in this case.

***(ii) Mass transport :-***

The deposition step in stripping analysis is usually facilitied by convective transport of the ions to the working electrode. This is achieved by electrode rotation or solution flow or by solution stirring. The overall sensitivity and precision are largely dependent on the effectiveness of the hydrodynamics which control the amount of the species plated during the deposition step. However, for any working electrode and convective transport used in stripping analysis, the same general deposition theory applies. However, under the forced convection conditions used in stripping analysis, both diffusion and convection contribute to the deposition current. This makes the solution of the corresponding hydrodynamic equation more complex [95].

***2- The rest period :-***

This is the period employed between the deposition and stripping steps. As the forced convection is stopped at the end of the deposition period, the deposition current drops almost to zero and a uniform concentration distribution is established very rapidly. The rest period also insures that the subsequent stripping step is performed in a quiescent solution, i.e., after convection in the solution has ceased. During the rest period, electro-deposition which is facilitated by the diffusion transport, is continued; this results in a non zero intercept in the peak current versus deposition time plot.

***3- The stripping step :-***

It consists of scanning the potential anodically or cathodically, linearly or in another potential-time wave form. When the potential reaches the standard potential of the using couple (metal-metal ion or organic compound in its

electrode and an equal opposite charge exists in the solution. The array of charged species and oriented dipoles existing at the surface is called the electrical double layer. When the potential of the working electrode is changed, a current must flow to charge or discharge the capacitor. This charging current is nonfaradic, because it flows in the absence of an accompanying redox reaction.

As this charging current exists even in highly purified solution, it usually limits the detectability of linear scan stripping procedures, for example, if the sensitivity of the peak current is increased by increasing the scan rate $\upsilon$ or the electrode surface. The faradic background component $i_{far}$ is composed of current that limit working potential range e.g:

In aqueous solution the cathodic potential range is limited by the reduction of hydrogen ions when these ions are the most readily species present:

$$2H^{+} + 2e^{-} \longrightarrow H_2$$

the resulting current is of then called "the hydrogen evolution current". The potential of this reaction can be expressed by:

$$E = E^{-}_{HZ/H^{+}} - 0.059\ pH \text{ ----------} (1.3)$$

Therefore, the more acidic the solution, the more positive is the potential of the hydrogen evolution current (59 mV shift per pH unit ).

Within the working potential range the faradic background current is resulted mainly from redox reactions of trace impurities that are almost inevitably present in the blank solution. Contributors include small amount of dissolved oxygen, heavy metal ions from the distilled water and impurities present in the salt used as the supporting electrolyte.