Nitro group containing pesticides are playing vital role in the agriculture field. These pesticides are widely used as pre- and post- emergent weed control agents (herbicides) for a wide variety of crops, namely corn, sorghum, wheat, rice, sugar cane and for fruits, vegetables and vineyards, consequently, they are found in river water, ground water^199-200 and soils.^201-203

Some nitro group containing pesticides were active components of formulations for the protection of plants. It is well known that nitro and especially dinitro compounds belong to the most effective but at the same time they act as toxic pesticides. The acute toxicity is not high in the case of the dinitro group containing pesticides. The LD₉₀ value is in the range 1000-10,000 mg/kg for various types of active compounds. Therefore, the discriminate use of these pesticides does not mean a serious hazard for human beings and animals. Nevertheless, some commercial formulations have successfully been made from the use in agriculture. On the other hand new types of effective pesticides are developed and verified from the nitro groups. Therefore, one of the main tasks in the analysis of pesticides and their formulations is the detailed study of properties of these pesticides. These studies are based on the monitoring in various levels of concentration of pesticides in the various environments.

Dinitro compounds were reported to exhibit significant antiproliferative and antiinfective activities against protozoan parasites including Leishmania spp,^206,207 Trypanosoma brucei^208 and the intraerythrocytic forms of Plasmodium falciparum.^209

Current methods of analysis for the analysis of pesticides containing nitro group compounds involve either liquid -liquid extraction^210 solid-phase extraction (SPE)^211 or supercritical fluid extraction (SCFE) and solid phase micro extraction (SPME).^212 etc. The main disadvantages of these methods were use of large quantities of often toxic and not eco-friendly solvents, the elaborate cleaning up time-
consuming procedures and the need for concentration of analytes before analysis.\textsuperscript{213,214}

Another detection method is commonly employed in the determination of dinitro herbicide residues by ECD in various environmental samples.\textsuperscript{215-219} The nitro groups in organic compounds are easily reduced at the mercury electrodes. This forms a basis for numerous polarographic and voltammetric studies.\textsuperscript{220-227} Whereas, the reduction of 2,6-dinitroanilines received some attention\textsuperscript{228,229} and reductive degradation of some pesticides containing dinitroaniline moiety was studied.\textsuperscript{230-232} The main electrode reaction responsible for voltammetric activity of nitro group pesticides was found to be the reduction of nitro group, which depends on the presence and nature of the substituent.

In the present investigation, agriculturally important nitro group containing pesticides like fluorodifen, acifluorfen and tecnazene were chosen to evaluate the electrochemical reduction behavior at the mercury electrodes and also to get clear information on the reduction mechanism of the nitro group present in their nuclei and the electrode kinetics concerned by using electrochemical techniques such as cyclic voltammetry, adsorptive stripping voltammetry, millicoulometry and controlled potential electrolysis. Adsorptive stripping voltammetric method was used for the determination of proposed compounds in various food and water samples of environmental importance.

**EXPERIMENTAL**

The description and use of the instrumentation, experimental procedures and the supporting electrolytes were described in Chapters II and III.

The samples of fluorodifen, acifluorfen and tecnazene were obtained from Riedel-de Haen, Germany, Dr. S. Erhenstorfer Augsburg
(Germany), Dow Agro, USA, respectively. The sample purity was tested by determining their melting points and TLC experiments.

RESULTS AND DISCUSSION

A. FLUORODIFEN

Fluorodifen (2-nitro-1-(4-nitrophenoxy)-4-(trifluoromethyl)benzene, Reg. No. 15457-05-3) belonging to the dinitrophenyl ether herbicides, which was widely employed for effective use on wide variety of crops including soybean, dry beans, dry peas, sunflowers, peanuts, pumpkins, squash and canola. Fluorodifen is the active ingredient in Preforan and Soyex herbicides, which primarily controls annual grasses such as foxtail spp., crab grass spp., and annual broad leaf weeds such as red root pigweed, kochia and black night shade.233,234

Several analytical techniques were developed in which mostly chromatographic techniques235,237 for the determination of the herbicide in various soil, crops, oil seeds and water samples. Smith et al.238 reported the granular formulations following surface active treatments. Fuyu Guan et al.239 was used SPME to separate dinitro group herbicides from formulations and vegetables.

EPA (Environmental Protection Agency), USA established the fluorodifen tolerances of 0.05 ppm in canola seed and sunflower seeds.240 Brown et al.241 evaluate the herbicides for pumpkin (cucurbita spp.). Singh et al.242 described the effect of pre-emergence applied herbicides on the symbiotic parameters and seed yield of soybean (Glycine max) (L) Merrill). Timothy Grey et al.243,244 reported the tolerances of cucurbits to the herbicides.

Electrochemical techniques especially voltammetric techniques have been considered as reliable analytical methods for the determination of trace levels of several dinitro phenoxy ether herbicides. In the present work, fluorodifen used to get more information on the reduction behaviour
and mechanism of nitro groups and electrode kinetics concerned by using cyclic voltammetry, differential pulse voltammetry and adsorptive stripping voltammetry. Adsorptive stripping voltammetric technique was employed for the determination in fluorodifen formulations and vegetables samples.

Characterisation of peaks

The electrochemical behavior of fluorodifen has been studied over the pH range from 2.0 to 12.0. A single well defined peak is observed throughout the pH range and this single peak is attributed to the simultaneous reduction of two nitro groups in eight electron process to the corresponding hydroxylamine groups. Typical cyclic voltammogram is shown in Fig. IV.1. In cyclic voltammetric experiments a small anodic peak, \( a_1 \) is observed on the reverse scan at the higher pH values (pH > 10.0) for fluorodifen. In the second scan, another small cathodic peak \( c_2 \) at more positive potentials than \( c_1 \) is noticed. The anodic peak \( a_1 \) may be due to the oxidation of hydroxylamine formed at \( c_1 \) to nitroso derivative and the cathodic peak \( c_2 \) may be attributed to the reduction of the nitroso derivative to the hydroxylamine again.\(^{244,246}\)

Cyclic voltammetric studies

The reduction process was found to be diffusion controlled and adsorption on the electrode surface in the buffer systems studied as evidenced from the linear plots of \( i_p \) vs \( v^{1/2} \) (Fig.IV.2) which were found to pass through origin. The irreversible nature of the peaks are seen from log plot analysis. Further, \( E_p \) values were observed to be shifted towards more negative values with increasing concentration of depolarizer. The current function \( (i_p / C v^{1/2}) \) in the above compound was found to be fairly constant with the scan rate \( (v) \) and the electrode process was purely diffusion controlled and adsorption on the electrode surface without any kinetic complications.
Identification of the product

Millieuoulometric technique was employed for the determination of number of electrons involved in the electrode process and was found to be eight in pH 6.0 for fluorodifen, which indicates the final product to be hydroxylamine. However, in basic medium (pH 8.0 to 12.0) only four electrons are involved in the electrode process which were ascribed to simultaneous reduction of two nitro groups. The formation of hydroxylamine was also evidenced from the appearance of anodic peak in cyclic voltammetric experiments. The anodic peak was attributed to the oxidation of hydroxylamine to nitroso derivative. If the hydroxylamine was not formed in the electrode process, the appearance of anodic peak will not arise. Controlled potential electrolysis experiment also agrees with the above results. CPE is carried out -0.6 V vs SCE at pH 6.0 for fluorodifen showed the product to be the corresponding hydroxylamine which is confirmed by I.R. spectral studies where the characteristic peaks for hydroxylamine groups (N-H stretch: 3430 cm⁻¹, N-H bend: 3100-3000 cm⁻¹) (Fig. IV.3).

Kinetic data

The values obtained for transfer coefficient, diffusion coefficient and heterogeneous forward rate constant for fluorodifen were given in Tables IV.1. The diffusion coefficient values evaluated from cyclic voltammetric technique were found to be in good agreement indicating the diffusion controlled. The diffusion coefficient values are decreasing with an increase in pH values. The reason may be due to the decrease in availability of protons with an increase in pH of the supporting electrolyte. The rate constant values were in general found to decrease with an increase in pH indicating that the electrode reaction tends to become more and more irreversible with change in pH.

Electrode mechanism

Based on the results obtained from all the techniques the electrochemical reduction of fluorodifen were shown below.
Differential pulse AdSV studies

Fig. IV.4 exhibits differential pulse adsorptive stripping voltammograms for 1 x 10^{-4} M fluorodiflun with HMDE. The systematic studies of the various experimental and instrumental parameters that affect the adsorptive stripping voltammogram response were carried out in order to establish the optimum conditions.

Effect of pH

The pH of a solution is a critical factor affecting both the rate and equilibrium state of the accumulation process and the rate of the electrode reaction. The influence of the pH on the DP-AdSV response was studied at hanging mercury drop electrode of the 1 x 10^{-4} M for fluorodiflun with accumulation time of 80 sec, between the pH ranges 2.0 to 12.0. It can be observed from Fig. IV.5 that the maximum peak currents are obtained with pH 6.0. When the pH was increased from 6.0 and 12.0, the peak potentials shifted towards more negative values.
Effect of accumulation potential

The accumulation potential also a major factor affecting the sensitivity. Fig. IV.6 shows that the optimal preconcentration potential condition was between 0.1 to -1.0 V for fluorodifen. The highest peak current was observed for an accumulation potential -0.6V. A gradual decrease in the peak hight was observed with the change in the potential to more negative or less negative potential than -0.6V. Thus an optimum pre-concentration potentials -0.6 V was used in the subsequent studies for the fluorodifen.

Effect of accumulation time

The adsorption behavior of fluorodifen was particular importance to be used to enhance the sensitivity of voltammetry. At first the stripping peaks with 't' increase linearly. It indicate that before adsorptive equilibrium was reached, the longer the accumulation time, the more fluorodifen was adsorbed and the larger was the peak current. However, after a specific period of accumulation time, the peak current tended to be leveled off, illustrating that adsorptive equilibrium of fluorodifen on the mercury electrode surface was achieved. Fig. IV.7 shows the effect of accumulation time on peak currents of 1 x 10^-4 M fluorodifen. The accumulation time of a 80 sec. was used for further studies.

Effect of scan rate

Adsortive stripping voltammograms obtained for increasing values of the scan rate showed the existence of a linear dependence of the peak current intensity on the scan rate between 40 to 80 mVs^-1. The peak currents were directly proportional to the scan rate indicating that the system was adsorption controlled.

Other experimental parameters such as temperature and ionic strength were optimized. The stripping peak currents were not modified when the temperature varied between 20-50°C. The room temperature 25°C was maintained for further studies. Several instrumental parameters which directly affect the voltammetric response were optimized, i.e., drop
size, stirring rate and pulse amplitude. The working conditions were decided up on medium drop size, 1500 rpm and 25mV. The stripping currents was not modified when varying the rest period, since it was found that 10 sec, was sufficient to allow for the formation of a uniform concentration of the analyte in the mercury drop.

Analysis

Investigated compound was found to exhibit well resolved peak at pH 6.0, and the sharp well resolved peak was chosen for quantitative studies. Peak currents were linear over the fluorodifen concentration range of $1.31 \times 10^{-8}$ M to $1.33 \times 10^{-8}$ M with lower detection limits of $1.09 \times 10^{-9}$ M. The lower detection limit was calculated using the expression

$$dI = 3 \text{ SD}/m$$

where SD was the standard deviation and m was the slope of the calibration plot. The relative standard deviation and correlation coefficients were found to be 1.25%, 0.996 respectively for 10 replicates.

Recommended analytical procedure

A stock solution ($1 \times 10^3$ M) of fluorodifen compound was prepared by dissolving required quantity of the compound in double distilled dimethylformamide (DMF). 1 ml of standard solution was transferred into a polarographic cell containing 9 ml of supporting electrolyte. Then the solution was purged with oxygen free nitrogen gas for 15 min. before recording the voltammogram. After recording the voltammogram, small increments (0.2ml) of standard solutions were added and voltammograms were recorded after each addition under similar conditions. In the present investigation the best precision was obtained at pH 6.0 with a drop time 2 sec., a pulse amplitude of 25mV, accumulation potential -0.6V, accumulation time 80 sec. and scan rate 45mVs$^{-1}$ for fluorodifen.

The above described procedure was successfully employed for the determination of fluorodifen in their formulations and vegetable samples.
The required quantity of fluorodifen (Preforan, Soyex) corresponding to a stock solution of concentration of $1 \times 10^{-3}$ M was accurately measured and transferred into a 100 ml calibrated flask and made up with dimethylformamide. A solution of approximately $1.0 \times 10^{-4}$ M was prepared by dilution of this stock solution with an appropriate universal buffer. The assay results for fluorodifen formulations are given in Table IV.2.

In the present investigation, vegetables such as potatoes and beans were chosen for the analysis of fluorodifen. Known amount of fluorodifen (preforan, soyex) was sprayed on potatoes and beans and left for 1-2 hours. The extracts were prepared by the treatment of a crushed sample with 100 ml of acetone. Then the extract was allowed to dry. The residue of fluorodifen was dissolved in DMF and transferred into a 50 ml volumetric flask. Then voltammograms were recorded in the same manner as described earlier. The results obtained using the DP-AdSV are shown in Table IV.3.

The selectivity of the proposed method for fluorodifen was tested in the presence of Cr (III), Cd (II), Zn (II), Ni (II), Co (II), Cr (VI), Cu (II), Pb (II), F, Br, and I. Only chromium (VI) copper (II) and lead (II) ions interfered seriously with the procedure proposed. The reduction of chromium (VI) to intermediate oxidation states between V and III at -0.6 V Vs SCE may be responsible for the influence of chromium (VI). Interference of copper (II) and lead (II) was attributed to a possible complex formation of these ions with the fluorodifen compound via the nitro group, which is then accumulated at the HMDE surface. There was no effect of these interferences on DP-AdSV signal.

Recoveries of fluorodifen (Preforan, Soyex) ranging from 98.20% to 99.80% for Preforan and 99.40% for 99.70% for Soyex have been made. For preforan the percentage of recoveries in vegetable samples ranging from 97.75% to 99.25% and 97.60% to 99.30% for potatoes and beans and
for soybeans the percentages of recoveries in vegetable samples ranging from 96.75% to 99.12% and 97.60% to 99.10% for potatoes and beans respectively.

**B and C. ACIFLUORFEN AND TECNAZENE**

Acifluorfen (5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid, Reg.No. 50594-66-6). Tecnazene (1,2,4,5-tetrachloro-3-nitrobensene, Reg. No. 117-18-0) are nitrophenyl ether herbicide and aromatic fungicide respectively. The acifluorfen was used to control broadleaf weeds and grasses in soybeans, peanuts, peas, and rice. It can be applied before or after crop emergence. It is especially effective against cocklebur, velvetleaf, common lambsquarters, monitoring glory, and jimsonweed. Acifluorfen selectively controls broadleaf weed when applied after soybeans have emerged.\(^\text{248}\) The activity of this compound was enhanced by sunlight. It may be toxic to some crop plants such as soybeans if mixed with fertilizers. Symptoms of acute exposure to acifluorfen are not recorded to humans. The oral LD\(_{50}\) of acifluorfen in male albino rats is 1,300 mg/Kg.\(^\text{249}\) In rabbits, the dermal LD\(_{50}\) of Blazer is 450 mg/Kg, while it is 2,000 mg/Kg for Tackle.\(^\text{250}\)

Tecnazene was used as a sprout inhibitor on stored potatoes and as a fungicide in smoke generators in greenhouses. It was unlikely to present an acute hazard in normal use, based on an oral LD\(_{50}\) in the rat of 1750 mg/Kg body weight, rapidly absorbed and metabolized in animals after oral administration; as the dose increases, larger amounts are passed unchanged in the faeces. For rats 750 mg/Kg diet, equivalent to 38 mg/Kg body weight per day; dog 15 mg/Kg body weight per day.\(^\text{261}\) Tecnazene has a low acute toxic to mammals.\(^\text{262}\) It is subjected to photodecomposition but not volatilisation at the soil surface.\(^\text{263}\)

The analysis of acifluorfen herbicide was performed by means of variety of techniques. Acifluorfen in soil and water is determined by using chromatograph equipped with a UV detector was developed\(^\text{264}\)
Mass spectrometry and (1H) nuclear magnetic resonance (NMR) are using for analysis of photochemical studies of acifluorfen. Water dissolved acifluorfen was irradiated and determined the numerous photoproducts by (1H)NMR and HPLC-MS/MS. Studied the kinetics of the inhibition of mitochondrial protoporphyrinogen oxidase (PPO) from liver and placenta of 3 mammalian species by the diphenyl ether herbicide acifluorfen. Studied the effect of the herbicide acifluorfen on microbial biomass and on hydrolytic capacity, and its persistence in a clay-loam soil before and after enrichment with glucose.

In the present investigation, acifluorfen and tecnazene have been selected to get more information on the reduction mechanism of nitro groups and electrode kinetics concerned using cyclic voltammetry, and adsorptive stripping voltammetry. Differential pulse adsorptive stripping voltammetry has also been employed to work out analytical procedure in trace level estimation of these herbicides in formulations, and grape juice samples.

Characterisation of peaks

The electrochemical behaviour of acifluorfen and tecnazene were examined over the pH range from 2.0 to 12.0. A single well defined peak is observed throughout the pH range for these two compounds in all above techniques. This single peak is attributed to the reduction of one nitro group in acifluorfen and tecnazene involve in four electron process to the corresponding hydroxylamine group. The typical cyclic voltammograms of these two compounds are shown in Fig.IV.8 and Fig. IV.9.

In cyclic voltammetric experiments, a small anodic peak (a1) has been observed in the reverse scan at higher pH values (pH >10.0) for the above two compounds. In the second scan, another small cathodic peak (c2) at more positive potentials than (c1) is noticed. The anodic peak (a1) may be due to the oxidation of hydroxylamine formed at (c1)to nitroso
derivative and the cathodic peak \((c_p)\) may be attributed to the reduction of the nitroso derivative to the hydroxylamine group again.

**Cyclic voltammetric studies**

The nature of the peak was found to be diffusion controlled and adsorbed on the electrode surface in the buffer systems taken, as shown by the linear plot of \(ip vs v^{1/2}\) (Fig. IV.10 and Fig. IV.11) which is found to pass through origin. The irreversibility of the electrode process was confirmed by log plot analysis of the peak. The variability of the peak potential with scan rate also indicates the irreversible nature of the electrode process. Further \(Ep\) values are observed to have shifted towards more negative values with increasing concentration of the depolarizer. The \(Ep\) values of acifluorfen and tecnazene were found to be dependent on pH and shift towards more negative values with the increase in pH of the buffer solutions, indicating proton involvement in the electrode process.

**Identification of the product**

Miticoulometry was employed to find out the number of electrons involved in the electrode process. It was found to be four electrons for the reduction of nitro group in acifluorfen and tecnazene both acidic and basic medium.

Controlled potential electrolysis (cpe) was carried out in a modified cell with mercury pool cathode, saturated calomel electrode and platinum wire as anode. This experiment is carried out at pH 6.0 at applied potential of -0.5V. After electrolysis, the reduced products are extracted with ether. The ethereal layer is evaporated on water bath and the products were identified as the corresponding hydroxylamine.

**Kinetic data**

The values for transfer coefficient \((\alpha)\), diffusion coefficient \((D)\) and heterogeneous forward rate constant \((k^*_{th})\) at various pH values in cyclic voltammetry technique were given in Table IV.4 and IV.5. The variation
of diffusion current and peak current with the pH of the supporting electrolyte influences the diffusion coefficient values. Because the slight variation in diffusion coefficient values with increase in pH for acifluorfen and tecnazene may be attributed to the decrease in the availability of protons.

The forward rate constant \( (k_{f,r}) \) values for acifluorfen and tecnazene were found to decrease with increase in pH. This trend shows that the electrode process becomes more irreversible with increase in pH of the solution.

With the above mentioned conclusions the following reaction scheme could be proposed which represents the chemical steps involving acifluorfen and tecnazene and its intermediates based on the experimental data were shown above:

**Electrode mechanism**

**Acifluorfen**

\[
\begin{align*}
\text{F}_2\text{C} & \quad \text{O} & \quad \text{C} \\
\text{Cl} & \quad \text{N} & \quad \text{OH} \\
\text{NO}_2 & \quad \text{O} & \quad \text{OH}
\end{align*}
\]

\[
\text{F}_2\text{C} \quad \text{O} \quad \text{C} \\
\text{Cl} \quad \text{N} \quad \text{O}
\]

\[
\begin{align*}
2e \quad \text{H}^+ & \quad \text{H}_2\text{O} \\
\end{align*}
\]

\[
\begin{align*}
\text{F}_2\text{C} & \quad \text{O} \\
\text{Cl} & \quad \text{N} \quad \text{OH} \\
\text{NH}_2\text{OH}
\end{align*}
\]
The electrochemical studies with hanging mercury drop electrode, using differential pulse adsorptive stripping voltammetry carried out for acifluorfen and tecnazene indicate that an adsorption process occur on the mercury electrode surface which can be used as an effective pre-concentration step prior to voltammetric measurement (Fig. IV.12 and Fig. IV.13). An exhaustive study of the dependence of adsorptive peak currents on pH, accumulation potential, accumulation time and scan rate were performed using $10^{-3}$ M acifluorfen and tecnazene solutions.

**Effect of pH**

Voltammograms were recorded at different pH values and a maximum intensity for pH 6.0 was obtained. At this pH acifluorfen and tecnazene yields a single well defined peaks (Fig. IV.14).

**Effect of accumulation potential**

The influence of the accumulation potential on the peak height of acifluorfen and tecnazene were studied from -0.2 to -1.0 V and a strong adsorption at -0.5 V (Fig. IV.15) was observed. Therefore, this potential
was used as the optimum accumulation potential for all the measurements.

**Effect of accumulation time**

Fig. IV.16 shows plots of cathodic peak current (ip) for DP-AdSV vs. accumulation time (t_{ac}) for 1 \times 10^4 M concentration of acifluorfen and tecnazene. At first, i_p increased linearly with t_{ac}, indicating that before adsorptive equilibrium was reached, the longer the preconcentration time, the more acifluorfen and tecnazene were adsorbed, and the larger the peak current. However, after a specific accumulation time, the peak current tended to level off, illustrating that adsorptive equilibrium of acifluorfen and tecnazene on the mercury electrode surface was achieved.

**Effect of scan rate**

The effect of scan rate (v) on the peak currents was evaluated for the adsorbed acifluorfen and tecnazene. The plot of log ip versus log v shows a linear relationship with a correlation coefficients of 0.997 and 0.998 for two compounds.

A linear relationship of the adsorption holds between the peak current and the concentrations of acifluorfen and tecnazene in the ranges from 8.4 \times 10^{-4} to 1.6 \times 10^{-4} M and 2.1 \times 10^{-4} M to 1.1 \times 10^{-4} M with a good precision and accuracy.

The influence of several instrumental parameters known to affect the differential pulse adsorptive stripping current response at the HMDE, such as mercury drop size, stirring rate, pulse amplitude, rest period and purge time were optimized. For this study, each variable was changed while the others were kept constant. The working conditions were decided upon medium drop size, 1500 rpm, 25 mV, 10 sec. The stripping currents were not modified when varying the rest period, since it was found that 10 sec. was sufficient to allow for the formation of uniform concentration of the analyte of acifluorfen and tecnazene in the mercury drop.
Other experimental parameter such as temperature was optimized. The stripping peak currents were not modified when the temperature varied between 20-50°C. The room temperature was chosen for further studies.

The optimal values of these parameters were then chosen from the study of the variation of the peak current (ip) of $1.0 \times 10^{-6}$ M acifluorfen and tecnazene in universal buffer of pH 6.0. The peak current of acifluorfen and tecnazene was found to increase linearly on increase with scan increment.

Analysis

In the present investigation differential pulse adsorptive stripping voltammetry (DP-AdSV) has been employed for the quantitative determination of acifluorfen and tecnazene in formulations, grain samples and grape juice samples. These two compounds were found to exhibit sharp and well resolved peaks at pH 6.0 and this peak is chosen for quantitative studies. Both standard addition and calibration methods are used. The peak heights are found to be linear over the concentration ranges of acifluorfen and tecnazene are $8.4 \times 10^{-6}$ to $1.6 \times 10^{-6}$ M and $2.1 \times 10^{-6}$ M to $1.1 \times 10^{-4}$ M with lower detection limits $2.47 \times 10^{-6}$ M and $1.5 \times 10^{-4}$ M respectively.

Recommended analytical procedure

A standard solution of acifluorfen and tecnazene (1 $\times 10^{-4}$ M) are prepared in dimethylformamide. One ml of standard solution was transferred into a polarographic cell and made up with 9 ml of supporting electrolyte (pH 6.0) and deoxygenated with N$_2$ gas for 10 min. subjected to voltammetry. After obtaining the voltammogram, a small increment of the standard solution (0.2 ml) of acifluorfen and tecnazene added to voltammetric cell and deaerated for 10 min. and voltammogram are recorded under similar conditions. In the same manner 10 voltammograms are recorded for 10 standard additions. The optimum conditions for analytical
determination were found to be at pH 6.0 with drop time of 2 sec., pulse amplitude of 25 mV and an applied potential of -0.5 V for both acifluorfen and tecnazene. The correlation coefficient and relative standard deviation (for 10 replicants) obtained with the recommended procedure are found to be 0.997, 1.14% for acifluorfen and 1.33, 1.48% for tecnazene.

The above developed analytical procedure was then applied to the determination of acifluorfen in formulations Acritet, Acifluorfen sodium salt and tecnazene in formulation Fusarex, TCNB. The required quantity of formulation corresponding to a 1.0 x 10⁻³ M stock solution was accurately measured and transferred into a 50 ml volumetric flask containing acetone. Standard solution of 1.0 x 10⁻⁶ M is prepared by dilution of this stock solution with universal buffer. Assay results for acifluorfen and tecnazene in formulations are given in Table IV.6 and IV.7.

Commercial grape juice samples were obtained from local markets. A 100 ml aliquot of juice samples were spiked with known amounts of tecnazene stock solution at different concentration levels. The extraction procedure was performed by using the procedure described as above. The residue was dissolved in acetonitrile and placed into a cell containing a suitable buffer solution. The standard addition method was used to estimate the compound in these samples. The recoveries obtained for tecnazene in juice samples were found to be comparatively high compared to those of the reference method and they are presented in Table IV.8.

There was no effect due to ingredients present in formulations of acifluorfen and tecnazene. In case of general formulations of above compounds causes no significant changes in the peak potential of the voltammetric reduction peaks obtained. Therefore, the proposed method does not involved the elaborate clean up procedures with the other methods.
Recoveries of acifluorfen formulations (acritet, acifluorfen sodium salt) ranging from 98.70% to 99.80% and 97.25% to 98.60% respectively. The recoveries of tecnazene (Fusarex, TCNB) in grape juice samples are ranging from 97.75% to 99.25% and 96.75% to 99.12% respectively.

The recoveries obtained for tecnazene formulations (Fusarex, TCNB) are in the ranging from 93.53% to 99.80% and 99.4% to 99.7%. From the results of the recoveries of Fusarex are high compared to remaining formulations of TCNB. Recoveries of tecnazene in spiked grape juice samples in range of 97.75% to 99.25% which indicates the high accuracy and reproducibility of the proposed differential pulse adsorptive stripping voltammetric method.

**Comparative Account**

In this Chapter highly effective and selective nitro group containing pesticides such as

A. Fluorodifen  
B. Acifluorfen  
C. Tecnazene

are chosen to understand the electrode kinetics and reduction mechanism concerned from the results of cyclic voltammetry, differential pulse adsorptive stripping voltammetry, controlled potential electrolysis and millicoulometry in the universal buffer solutions of pH ranging from 2.0-12.0. Analytical procedures are described for their monitoring in various food samples by employing DP-AdSV method.

From the experimental results obtained for the electrochemical reduction behaviour of the above said compounds, the total number of peaks observed is found to be one. All the three compounds studied at pH range 2.0 to 12.0 (both acidic and alkaline medium). In fluorodifen single peak attributed to the simultaneous reduction of nitro groups to the corresponding hydroxylamine groups with an uptake of eight electrons. In case of acifluorfen and tecnazene single well defined peak is attributed
to the reduction of nitro group in four electron process to the corresponding hydroxylamine group. In cyclic voltammetry, a small anodic peak was observed in the reverse scan. It is quite likely that a nitroso compound was formed, whose movement at the electrode surface may be responsible for the anodic peak at higher pH values (pH>10.0) for these two compounds.

The reduction of nitro group is found to be facile in the above said compounds, apparently due to the orientation of different substituents to the surrounding nitro group. The peak potentials of the three compounds show that fluorodifen in comparison to acifluorfen and tecnazene are reduced at less negative potentials. This may be due to the presence of trifluoromethyl group in fluorodifen. However, due to presence of alkyl groups which make hindrance for the reduction may result only hydroxylamine as the end product in acifluorfen and tecnazene. In case of tecnazene the one nitro group is reduced to the corresponding hydroxylamine in acidic medium and in alkaline medium. Thus the case of reduction can be explained based on the structure of the pesticide, especially the position of the nitro group in the aromatic ring and substituents in the benzene ring.

The electrochemical reduction behavior of all three compounds in the present investigation is found to be irreversible, adsorption on the electrode surface and diffusion controlled in nature in all the techniques over the pH range 2.0 to 12.0. Ep values of three compounds are found to be pH dependent and are observed to shift cathodically with an increase in the pH of the electrolyte indicating the proton involvement on the reduction process. An increase in the percentage of solvent (DMF) to the test solution, peak potentials are seem to be shifted towards more negative values with a simultaneous decrease in diffusion current in the three investigated compounds. This may be explained as due to the possible adsorption of the solvent molecules on the surface of the working electrodes and also due to the increase in viscosity of the medium.
pH of the supporting electrolyte is found to influence the diffusion current and peak current values, which in turn changes the diffusion coefficient values in the same manner in all the compounds. The diffusion coefficient values obtained for the investigated compounds are found to be in good agreement in all the techniques. The heterogeneous forward rate constant values for the reduction of nitro groups in these compounds are observed to be high, since the nitro group reduction it facile which is evidenced from the less negative reduction potentials obtained. The rate constant values are seen to decrease gradually with increase in pH of the solution in all the techniques for these compounds.

By employing DP-AdSV, analytical procedures are described for the quantification of the above compounds. Both standard addition and calibration methods are utilized for the estimation of these agriculturally important pesticides in their formulations, grains, vegetables and fruit juice samples. The optimal experimental conditions are found to be a drop time of 2 sec. and pulse amplitude of 25 mV for the three compounds. In agricultural formulations, the recoveries in the range from 96.33% to 99.85% are obtained for these compounds. Similarly, 98.20% to 99.00%, 97.88% to 99.20% recoveries of flourofen in vegetable samples (potatoes, beans), 91.04% to 91.56%, 89.34% to 90.89% of tecnazene in vegetable samples (potato, soy bean), 97.75% to 98.33%, 96.75 to 99.12 (fusarex, TCNB) of tecnazene in grape juice samples are obtained with the proposed DP-AdSV method, which indicates the accuracy and reproducibility of DP-AdSV method.
Table IV.1: Cyclic voltammetric data of fluorodifen

Concentration: $1 \times 10^{-4}$; Scan rate: $45 \text{mVs}^{-1}$

<table>
<thead>
<tr>
<th>pH of the supporting electrolyte</th>
<th>$-E_p$ / V</th>
<th>$i_p$ / $\mu$A</th>
<th>$a_n$</th>
<th>$D \times 10^4$ / cm$^2$ s$^{-1}$</th>
<th>$K^o f, h$ / cm s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>0.61</td>
<td>7.2</td>
<td>1.65</td>
<td>2.17</td>
<td>$3.22 \times 10^4$</td>
</tr>
<tr>
<td>4.0</td>
<td>0.67</td>
<td>5.7</td>
<td>1.62</td>
<td>1.83</td>
<td>$3.82 \times 10^4$</td>
</tr>
<tr>
<td>6.0</td>
<td>0.73</td>
<td>5.9</td>
<td>1.67</td>
<td>1.72</td>
<td>$2.04 \times 10^{11}$</td>
</tr>
<tr>
<td>8.0</td>
<td>0.61</td>
<td>6.4</td>
<td>1.61</td>
<td>3.93</td>
<td>$6.82 \times 10^{10}$</td>
</tr>
<tr>
<td>10.0</td>
<td>0.74</td>
<td>5.7</td>
<td>1.63</td>
<td>3.14</td>
<td>$7.28 \times 10^{12}$</td>
</tr>
<tr>
<td>12.0</td>
<td>0.85</td>
<td>5.4</td>
<td>1.62</td>
<td>3.05</td>
<td>$9.14 \times 10^{13}$</td>
</tr>
</tbody>
</table>
Table IV.2: Determination of fluorodifen in formulations by DP-AdSV
Pulse amplitude: 25 mV  Drop time: 2 sec.

<table>
<thead>
<tr>
<th>Name of the formulation</th>
<th>Labelled amount (mg)</th>
<th>Amount found (mg)</th>
<th>Recovery (%)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preforan</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>4.91</td>
<td>98.20</td>
<td>0.057</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>9.98</td>
<td>99.80</td>
<td>0.080</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>14.77</td>
<td>98.53</td>
<td>0.030</td>
</tr>
<tr>
<td>Soyex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>4.97</td>
<td>99.40</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>9.97</td>
<td>99.70</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>14.95</td>
<td>99.66</td>
<td>0.077</td>
</tr>
</tbody>
</table>

Table IV.3: Recoveries of fluorodifen added to potatoes and beans
Pulse amplitude: 25 mV  Drop time: 2 sec.

<table>
<thead>
<tr>
<th>Name of the formulation</th>
<th>Amount added (mg)</th>
<th>Amount found (mg)</th>
<th>Average Recovery (%)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Potatoes</td>
<td>Beans</td>
<td>Potatoes</td>
<td>Beans</td>
</tr>
<tr>
<td>Preforan</td>
<td>4.0</td>
<td>5.0</td>
<td>3.93</td>
<td>4.88</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>10.0</td>
<td>7.82</td>
<td>9.93</td>
</tr>
<tr>
<td></td>
<td>12.0</td>
<td>15.0</td>
<td>11.91</td>
<td>14.85</td>
</tr>
<tr>
<td>Soyex</td>
<td>4.0</td>
<td>5.0</td>
<td>3.87</td>
<td>4.88</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>10.0</td>
<td>7.93</td>
<td>9.91</td>
</tr>
<tr>
<td></td>
<td>12.0</td>
<td>15.0</td>
<td>11.80</td>
<td>14.82</td>
</tr>
</tbody>
</table>

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Table IV.4: Typical cyclic voltammetric data of Acifluorfen
Concentration: 0.5 mM  Scan rate: 45 mV s\(^{-1}\)

<table>
<thead>
<tr>
<th>pH of the supporting electrolyte</th>
<th>(-\frac{E_p}{V})</th>
<th>(i_p) (\mu A)</th>
<th>(\alpha n_e)</th>
<th>(D \times 10^4) (cm^3 s^{-1})</th>
<th>(K^\circ f, h) (cm s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>0.94</td>
<td>7.84</td>
<td>0.89</td>
<td>6.74</td>
<td>8.39 \times 10^4</td>
</tr>
<tr>
<td>4.0</td>
<td>1.00</td>
<td>7.87</td>
<td>0.83</td>
<td>6.45</td>
<td>4.53 \times 10^{10}</td>
</tr>
<tr>
<td>6.0</td>
<td>0.74</td>
<td>6.60</td>
<td>0.79</td>
<td>5.97</td>
<td>5.27 \times 10^4</td>
</tr>
<tr>
<td>8.0</td>
<td>1.21</td>
<td>6.73</td>
<td>0.74</td>
<td>5.88</td>
<td>8.32 \times 10^4</td>
</tr>
<tr>
<td>10.0</td>
<td>1.37</td>
<td>6.19</td>
<td>0.71</td>
<td>5.47</td>
<td>8.83 \times 10^4</td>
</tr>
<tr>
<td>12.0</td>
<td>1.45</td>
<td>5.86</td>
<td>0.62</td>
<td>5.19</td>
<td>7.23 \times 10^4</td>
</tr>
</tbody>
</table>

Table IV.5: Typical cyclic voltammetric data of tecnazene
Concentration: 0.5 mM  Scan rate: 45 mV s\(^{-1}\)

<table>
<thead>
<tr>
<th>pH of the supporting electrolyte</th>
<th>(-\frac{E_p}{V})</th>
<th>(i_p) (\mu A)</th>
<th>(\alpha n_e)</th>
<th>(D \times 10^4) (cm^3 s^{-1})</th>
<th>(K^\circ f, h) (cm s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>0.72</td>
<td>9.4</td>
<td>0.72</td>
<td>4.03</td>
<td>6.94 \times 10^{13}</td>
</tr>
<tr>
<td>4.0</td>
<td>0.85</td>
<td>9.6</td>
<td>0.83</td>
<td>3.62</td>
<td>2.30 \times 10^{13}</td>
</tr>
<tr>
<td>6.0</td>
<td>0.73</td>
<td>6.8</td>
<td>0.93</td>
<td>2.68</td>
<td>9.23 \times 10^{14}</td>
</tr>
<tr>
<td>8.0</td>
<td>1.14</td>
<td>9.0</td>
<td>1.37</td>
<td>2.45</td>
<td>2.62 \times 10^{13}</td>
</tr>
<tr>
<td>10.0</td>
<td>1.30</td>
<td>8.8</td>
<td>1.45</td>
<td>2.23</td>
<td>4.28 \times 10^{17}</td>
</tr>
<tr>
<td>12.0</td>
<td>1.44</td>
<td>7.0</td>
<td>1.54</td>
<td>2.10</td>
<td>3.23 \times 10^{14}</td>
</tr>
</tbody>
</table>
### Table IV.6: Estimation of acifluorfen in pure form and in formulations

Pulse amplitude: 25 mV
Drop time: 2 sec

<table>
<thead>
<tr>
<th>Name of the formulation</th>
<th>Labelled Amount (mg)</th>
<th>Amount found (mg)</th>
<th>Average Recovery (%)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrifluorfen sodium salt</td>
<td>5.0</td>
<td>4.94</td>
<td>98.80</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>9.87</td>
<td>98.70</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>14.98</td>
<td>99.86</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>19.45</td>
<td>97.25</td>
<td>0.025</td>
</tr>
<tr>
<td>Acritet</td>
<td>5.0</td>
<td>4.93</td>
<td>98.60</td>
<td>0.075</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>9.84</td>
<td>98.40</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>14.70</td>
<td>98.00</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>19.45</td>
<td>97.25</td>
<td>0.025</td>
</tr>
</tbody>
</table>

### Table IV.7: Determination of tecnazene in formulations

Pulse amplitude: 25 mV
Drop time: 2 sec.

<table>
<thead>
<tr>
<th>Name of the formulation</th>
<th>Labelled amount (mg)</th>
<th>Amount found (mg)</th>
<th>Recovery (%)*</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusarex</td>
<td>5.0</td>
<td>4.91</td>
<td>98.20</td>
<td>0.057</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>9.98</td>
<td>99.80</td>
<td>0.080</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>14.03</td>
<td>98.53</td>
<td>0.030</td>
</tr>
<tr>
<td>TCNB</td>
<td>5.0</td>
<td>4.97</td>
<td>99.40</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>9.97</td>
<td>99.70</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>14.95</td>
<td>99.66</td>
<td>0.077</td>
</tr>
</tbody>
</table>
Table IV.8: Recoveries of tecnazene added to grape juice

Pulse amplitude: 25 mV  
Drop time: 2 sec.

<table>
<thead>
<tr>
<th>Name of the formulation</th>
<th>Amount added (mg)</th>
<th>Amount found (mg)</th>
<th>Average Recovery (%)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>grape juice</td>
<td>grape juice</td>
<td>grape juice</td>
<td>grape juice</td>
</tr>
<tr>
<td>Fusarex</td>
<td>4.0</td>
<td>3.93</td>
<td>98.25</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>7.82</td>
<td>97.75</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td>12.0</td>
<td>11.91</td>
<td>99.25</td>
<td>0.072</td>
</tr>
<tr>
<td>TCNB</td>
<td>4.0</td>
<td>3.87</td>
<td>96.75</td>
<td>0.072</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>7.93</td>
<td>99.12</td>
<td>0.033</td>
</tr>
<tr>
<td></td>
<td>12.0</td>
<td>11.80</td>
<td>98.33</td>
<td>0.018</td>
</tr>
</tbody>
</table>
Fig. IV.1. Typical cyclic voltammogram of fluorodifen for an accumulation time of 80 sec at HMDE, accumulation potential: -0.6V; Rest time: 10 sec; stirring rate: 1500 rpm; Scan rate: 45 mV s\(^{-1}\); Concentration: 1x10\(^{-6}\) M; pH: 8.0.
Fig. IV.2: $i_p$ Vs $V^{1/2}$ plots of fluorodifen, Concentration: 0.5 mM:
Scan rate: 45 mVs$^{-1}$
Fig. IV.3. I.R. Spectrum of the reduction product of fluorodifen.
Fig. IV.4. Typical differential pulse adsorptive stripping voltammogram of fluorodifen for HMDE (b); blank solution (pH 6.0) (a); accumulation time of 80 sec at HMDE, accumulation potential: -0.6V; rest time: 10 sec; stirring rate: 1500 rpm; scan rate: 45 mVs⁻¹; concentration: 1x10⁻⁴M.
Fig. IV.5. Effect of pH on fluorodifen solutions at HMDE:
accumulation time: 80 sec; accumulation potential: -6.0V;
rest time: 10 sec; stirring rate: 1500 rpm; scan rate: 45 mVs\(^{-1}\); pulse amplitude: 25mV.
Fig. IV.6. Effect of potential on the DP-AdSV response at HMDE; accumulation time: 80 sec; rest time: 10 sec; stirring rate: 1500 rpm; scan rate: 45 mVs⁻¹; pulse amplitude: 25 mV.
Fig IV.7. Effect of accumulation time of the DP-AdSV response of fluorodifen at HMDE; accumulation potential: -0.6V; rest time: 10 sec; stirring rate: 1500 rpm; scan rate: 45 mVs⁻¹; pulse amplitude: 25 mV.
Fig. IV.8. Typical cyclic voltammogram of acifluorfen for an accumulation time of 80 sec at HMDE; accumulation potential: -0.5 V; rest time: 10 sec; stirring rate: 1500 rpm; Scan rate: 45 mVs⁻¹; Concentration: 1x10⁻⁶ M; pH: 6.0.
Fig.IV.9. Typical cyclic voltammogram of tecnazene for an accumulation time of 80 sec at HMDE; accumulation potential: -0.5V; rest time: 10 sec; stirring rate: 1500 rpm; Scan rate: 45 mVs⁻¹; Concentration: 1x10⁻⁶ M; pH: 6.0.
Fig. IV.10. $I_p$ vs $V^{1/2}$ plots of acifluorfen, Concentration: 0.5 mM:
Scan rate: 45mVs$^{-1}$.
Fig IV.11. $I_p$ vs $V^{1/2}$ plots of tecnazene, concentration: 0.5 mM; scan rate: 45 mVs$^{-1}$. 

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Fig. IV.12. Typical differential pulse adsorptive stripping voltammogram of acifluorfen at HMDE (b); blank solution (pH 6.0) (a); accumulation time of 80 sec at HMDE; accumulation potential: -0.5V; rest time: 10 sec; stirring rate: 1500 rpm; scan rate: 45 mV s⁻¹; concentration: 1x10⁻⁴ M.
Fig.IV.18. Typical differential pulse adsorptive stripping voltammogram of tecnazene at HMDE (b); blank solution (pH 6.0) (a); accumulation time of 80 sec at HMDE; accumulation potential:-0.5V; rest time: 10 sec; stirring rate: 1500 rpm; scan rate: 45 mVs⁻¹; concentration: 1x10⁻⁶ M.
Fig. IV.14. Effect of pH on acifluorfen and tecnazene solutions at HMDE; accumulation time: 80 sec; accumulation potential: -0.5 V; rest time: 10 sec; stirring rate: 1500 rpm; scan rate: 45 mVs⁻¹; pulse amplitude: 25 mV.
Fig.IV.15. Effect of accumulation potential on the DP-AdSV response at HMDE; accumulation time: 80 sec; rest time: 10 sec; stirring rate: 1500 rpm; scan rate: 45 mVs\(^{-1}\); pulse amplitude: 25mV.
Fig.IV.16. Effect of accumulation time of the DP-AdSV response at HMDE; accumulation potential: -0.5V; rest time: 10 sec; stirring rate: 1500 rpm; scan rate: 45m Vs\(^{-1}\); pulse amplitude: 25 mV.