

Nano Scale Potentiometric and Spectrophotometric Assays for 2,4-Dichlorophenoxyacetic Acid

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Abstract

The aim of this work was to investigate the ability of silicate wire ion selective electrode to measure 2,4 dichlorophenoxyacetic acid (2,4-D). The slope of the electrode was 56.2 mV decade⁻¹ in the range from 10⁻⁶ to 10⁻² M with lower limit of detection 8.3 × 10⁻⁷ M. The electrodes were successfully applied to determination of 2,4-D by separate and standard addition potentiometry. The electrode was used to detect the effect of pH and temperature on 2,4-D placed on baby watercress plant grown in the clay soil and the residue after five day from addition. Leaf chlorophylls quantity measured utilize spectrophotometric or colorimetric assay following extraction of pigments of watercress by ethanol. This work was carried at 286, 440, 662 nm. Beer's law was obeyed in the concentration range of 2.21 mg/ml:2.21 µg/ml. The limits of detection were 2.21 µg/ml.

Keywords: 2,4-Dichlorophenoxyacetic acid; Potentiometric; Spectrophotometric; Wire ion-selective electrodes, Watercress; Chlorophyll

Introduction

2,4-D is one of the most widely used herbicides in the world and is characterized as low-cost, quite efficient even at low concentrations, and moderately hazardous (class II) according to the World Health Organization [1] Although this herbicide easily moves through the soil, its leaching into the groundwater can be minimized due to the degradation by microorganisms and also the absorption by plants. Its primary route of degradation in the environment is by microorganisms, which increases with temperature, humidity, pH, and organic matter content [2,3]. Microbial degradation is a possible route for the breakdown of 2,4-D, but it is very dependent on the characteristics of the water. Laboratory studies have shown that in warm, nutrient rich water that has been previously treated with 2,4-D microbial degradation can be a major factor for dissipation [4]. Residues of 2,4-D and its salts or esters in water are commonly measure by extraction, chemical derivatization, separation by gas-liquid chromatography and electron capture detection [4]. Other methods used in the determination of 2,4-D residues include high-performance liquid and thin-layer chromatography [5-7].

Several methods have been reported for the analysis of 2,4-D in food and environmental samples. These methods include gas chromatography (GC) [8-10]. High-Performance Liquid Chromatography (HPLC) and UV-spectrophotometry [11,12]. However the chromatographic techniques are expensive and not available in many quality laboratories worldwide. Spectrophotometry is probably the most convenient analytical technique for routine analysis because of its inherent simplicity, low cost and wide availability in quality control laboratories [13].

Experimental Section

Materials and methods chemicals

All chemicals used were of analytical-reagent grade. 2,4 Dichlorophenoxyacetic acid (Fluka AG) 95%, Sodium silicate (99%), Potassium chloride (99%), Sodium hydroxide (98%), Barium nitrate (98.5%), Ferrous sulphate (98%), L-Glutamic acid (99%), L-Ascorbic acid (99%), Maltose, D-Glucose (extra pure), Acrylamide (99%) and 1,2 Dichloroethane (98%) were provided by (LOBA Chemie) Fluka,

Switzerland, Hydrochloric acid (37%) from (Scharau). Ethanol (96%), from HAYMAN and Methanol (99.8%) from AnalaR Distilled water was used to prepare all solutions.

Instrumentation

The electrochemical measurements were carried out with HANNA instrument 211 (EZODO) for measuring pH. Sensitive balance model AG204 (METTLER TOLEDO) was used for measurements. Spectral measurements were carried out by using a single beam UV (OPTIMA) SP-SPECTROPHOTOMETER, with quartz cells of 1 cm optical path length.

Stock standard solution of 2,4-D

An accurately 0.01 M solution was prepared by dissolving 0.212 g of 2,4-D standard in 25% ethanol, transferred into a 100 mL volumetric flask and diluted to the mark with 25% ethanol and mixed well. This stock solution was further diluted with 25% ethanol to obtain working solutions in the ranges of 10⁻³:10⁻¹¹ M, reservation solutions in brown bottles inside the refrigerator.

Construction of wire ion selective electrode

Membrane was prepared by mixing well of 96% sodium silicate, 1% active coal, 3% dioctylephthalate with 1 cm of fusible polyethylene tube. The mixture was poured into 3 cm diameter petri dish with 10 ml tetrahydrofuran (THF). A pure silver wire electrode was dipped in the coating membrane solution several time, allow the film to dry for about five min repeat the process until 2.0 mm diameter. Wire electrode was soaked about 3 h in 1 × 10⁻³ M of 2,4-D. All potentiometric determination were performed using the analytical cell, Ag /membrane/2,4-D test solution//Reference electrode.

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Received June 28, 2015; Accepted July 14, 2015; Published July 21, 2015

Citation: El-Beshlawy MM (2015) Nano Scale Potentiometric and Spectrophotometric Assays for 2,4-Dichlorophenoxyacetic Acid. J Anal Bioanal Tech 6: 257 doi:10.4172/2155-9872.1000257

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Electrode calibration

Connect the wire electrode to the pH meter in the presence of reference electrode. The calibration of the electrode was preceded using standard solutions of 2,4-D ranging from 10^{-7} : 10^{-2} M.

Separate and spiking addition method was used by the sequence from low to a higher concentration. The measured potential was plotted against logarithm of 2,4-D concentration after one min (Figure 1). The electrode was washed with distilled water and dried with tissue paper and back to base line by immersing in water between measurements, stored in a dark place immersed in 10^{-5} M.

Response times

Calibration curve was measured from the lower (10^{-7} M 2,4-D) to the higher concentrated (10^{-2} M of 2,4-D) solutions the time traces of the calibration curves of silicate electrode was represented in Figure 2.

Selectivity

Selectivity coefficients $K^{pot}_{2,4-D}$ of the electrodes towards different species were determined by the standard addition technique method [14] in which the following equation was applied

$$K^{pot} = (a^{\prime} A - aA) / aB$$

where $a^{\prime} A$ known activity of primary ion, aA fixed activity of primary ion and aB activity of interfering ions. The results are appear by potting mV in the presence of different interference (Figure 3).

Effect of pH

The effect of pH of the potential of electrode was investigated. The potential was measured at a 2,4-D concentration solution (10^{-5} M) from the pH value of 3 up to 9. The solution was acidified by the addition of very small volumes of 0.2 N HCl then the pH value was increased gradually using 0.2 N NaOH for each pH value, two pH/mV meters were used to measure the potential. The potential was recorded and plotted against different pH values (Figure 4).

Analytical application

Growing watercress: Watercress seeds were germinated in 250 gm clay dry soil with about 3-10 seeds per pot, after two weak. The temperature of the plants was maintained between 20-25°C.

Watercress treatment: A total of 20 pots were used to grow the watercress plants, two pots didn't contain 2,4-D. Stock solution of 2.21 μ g /ml of 2,4-D was prepared in 25% ethanol as solvent eighteen pots were treated by 50 ml of the previous stock solution for each pot after adjust number of watercress plants (1, 2, 3, 5, 8,..) and, the pH (2.5-8) and temperature of solutions (-5, 15, 27, 43), plants are irrigated daily with distilled water.

Residual of 2,4-D: After two, three and five days from adding pesticides on the pot of watercress plants. 40 ml distilled water are added to each pot. The calibration curve was used to determine the concentration of 2,4-D in each pot plant solution above, all measurements was recorded after one minute of electrode immersing. Effect of pH and temperature on the residual was drawing in Figures 5 and 6.

Spectrophotometric determination of 2,4-D

Selection of solvent: It was important to see the effects of various solvents on the photodegradation of 2,4-D. After assessing the solubility of 2,4-D in different solvent as methanol, ethanol, 25% ethanol and 2,4

dichloroethane. 25% ethanol has been selected as common solvent for developing spectral characteristics, 0.00221 ng /ml solution of 2,4-D present in each solution. The absorbencies are plotted against the different solvent at 286 nm (Figure 7). It has been observed the absorption within half an hour of the beginning of the preparations in each solvent. Blank solution was the solvent.

Beer's Law concentration range: The stock solution were suitable diluted with 25% ethanol to get concentration range from 0.00221 g ml^{-1} -0.0221 ng/ml for 2,4-D. The absorbance was measured at 286 nm. Standards of the compound of interest are made, and their absorbance and concentration values are used to create a standardization graph (Figure 8). From the calibration curve 2,4-D obey Beer's law between 2.21 mg/ml to 2.21 μ g/ml.

Photo degradation of 2,4-D by UV Light: The cuvet was filled within about 1.5 ml of the top with the blank and 10^{-3} M of 2,4-D solution. Wipe the outside of each cuvet gently with a lint-free tissue to remove any fingerprints or solution that may be on the surface. Adjust the wavelength to 286 nm, the absorbance of 10^{-3} M of 2,4-D was determined and plotted against time (Figure 9).

Effect of 2,4-D on watercress leaf chlorophyll Extract: Young baby-leaf watercress were acquired directly from organic local farmers in region of South Saudi Arabia. Cut of 7 g of watercress leaf, rinse the leaves with distilled water and pat dry. Tear up the leaves and place

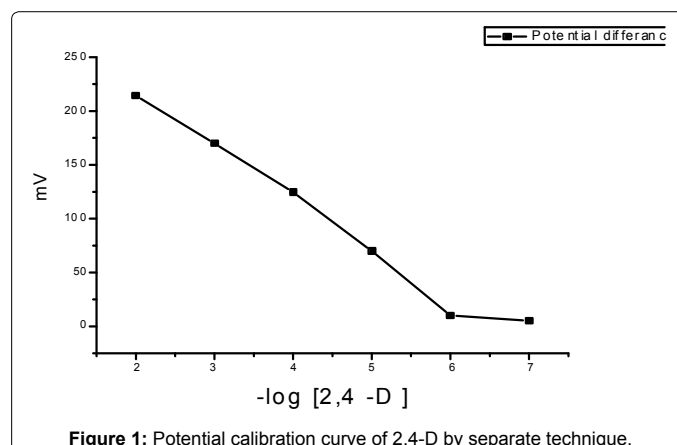


Figure 1: Potential calibration curve of 2,4-D by separate technique.

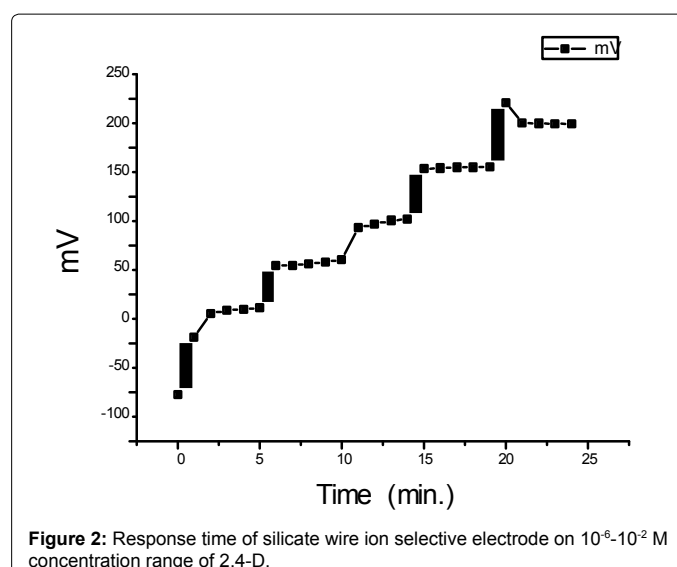
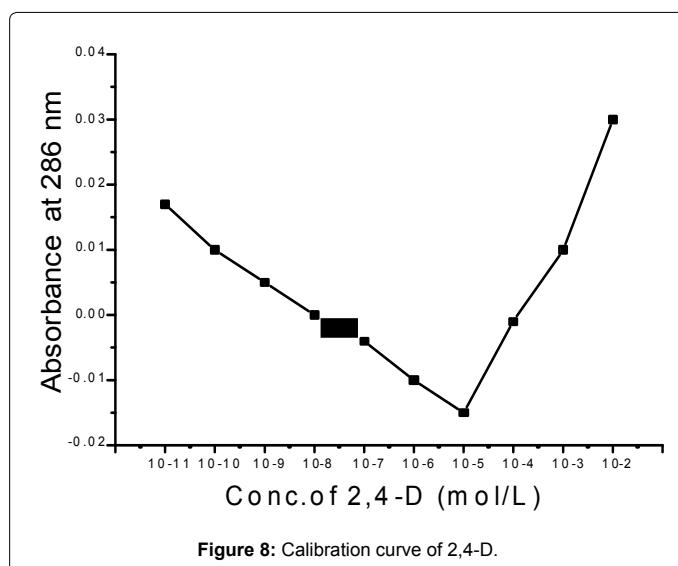
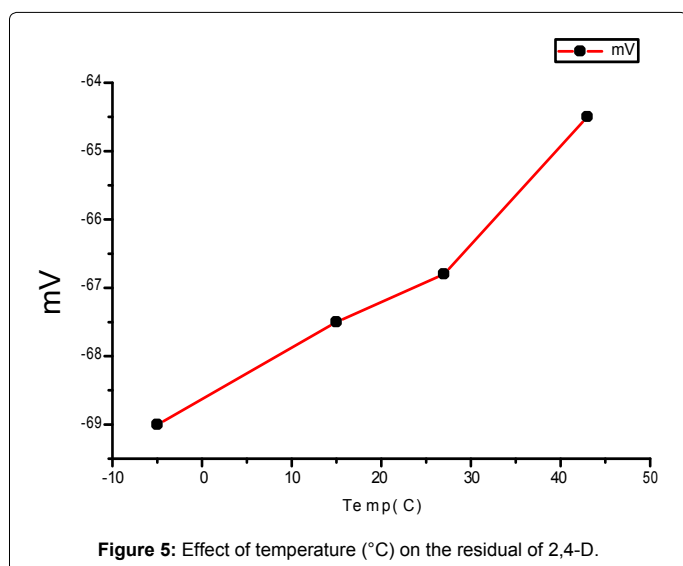
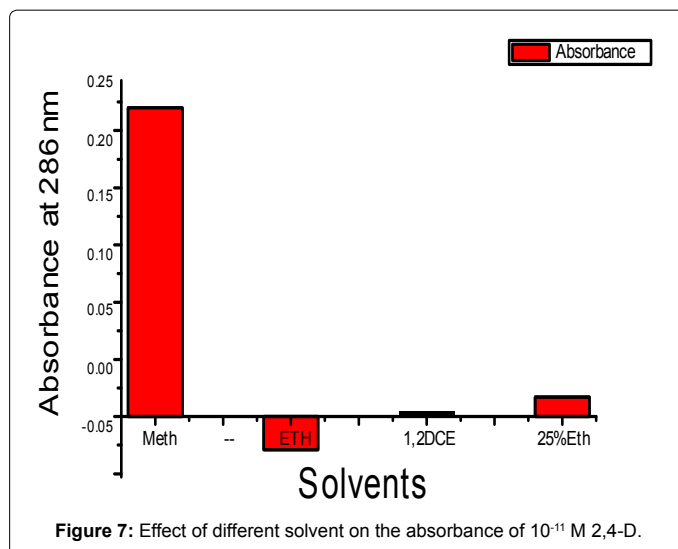
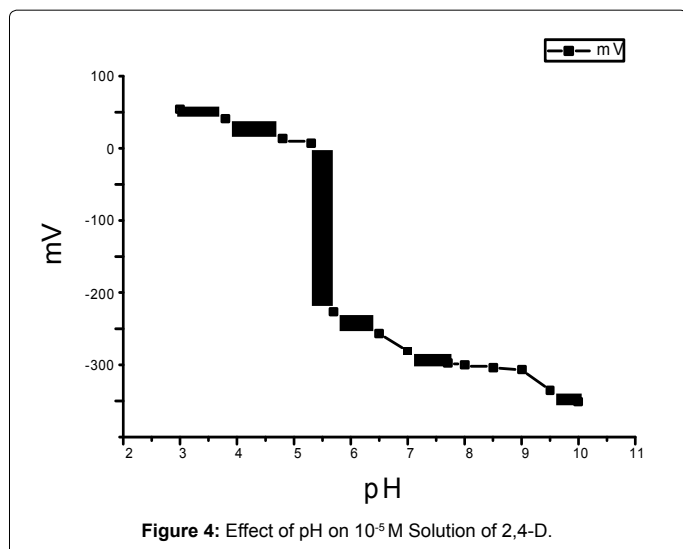
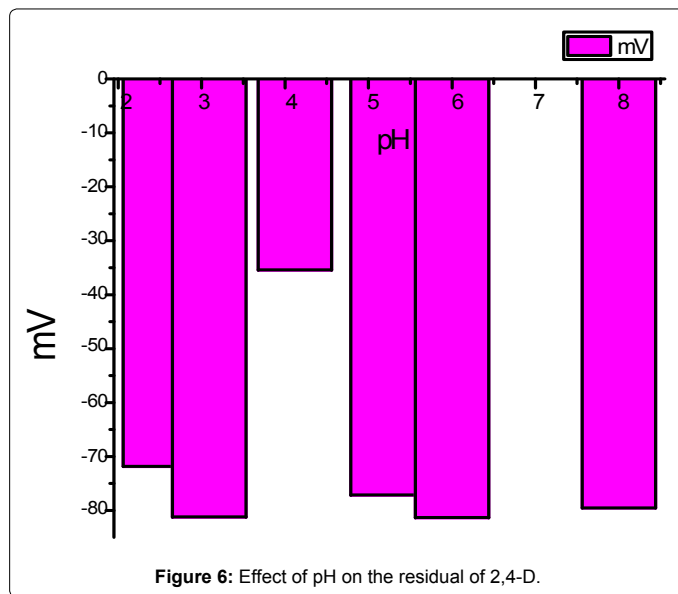
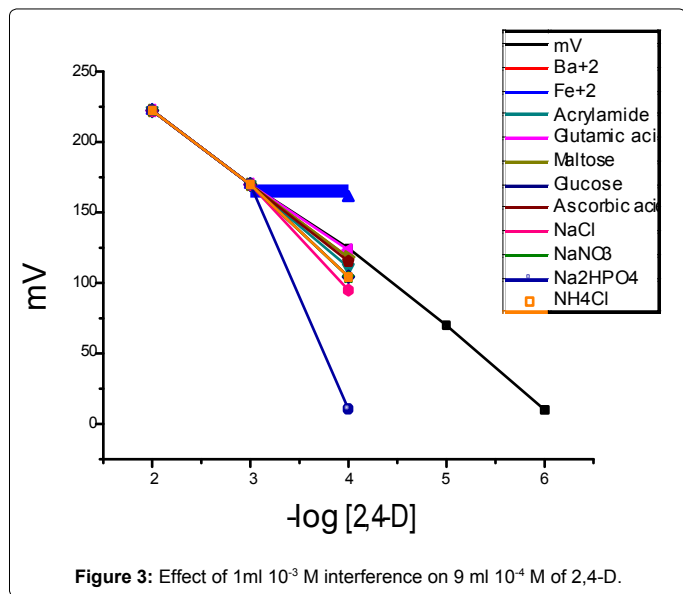


Figure 2: Response time of silicate wire ion selective electrode on 10^{-6} - 10^{-2} M concentration range of 2,4-D.



them in a 50 ml ethanol half an hour. Ethanol is a water-miscible solvent, removes all pigments from soft-leaved. The blank solution was prepared by 1 ml of chlorophyll extract and 11 ml distilled water and sample stock solutions were prepared by mixing aliquots of different concentration (10^{-11} , 10^{-5} and 10^{-3}) of 2,4-D were precisely pipette into 10 ml distilled water and 1 ml chlorophyll extract. The absorbance of solutions was measured at 286, 440, 662 nm and plotted against different concentration of 2,4-D in sample solutions (Figure 10).

Time considerations: Effect of time on the absorbance of watercress leaf chlorophyll extract with 1 ml different concentration of (10^{-11} , 10^{-5} and 10^{-3} M) of 2,4-D solutions was determined and plotted (Figure 11).

Result and Discussion

2,4-D was classified among the phenoxy acid herbicides MCPA as a class-2B carcinogen possibly carcinogenic to humans. The movement of auxin, (2,4-D) is a common herbicide,. Denmark, Norway, Kuwait and the Canadian provinces of Québec and Ontario, 2,4-D use is severely restricted in the country of Belize [15].

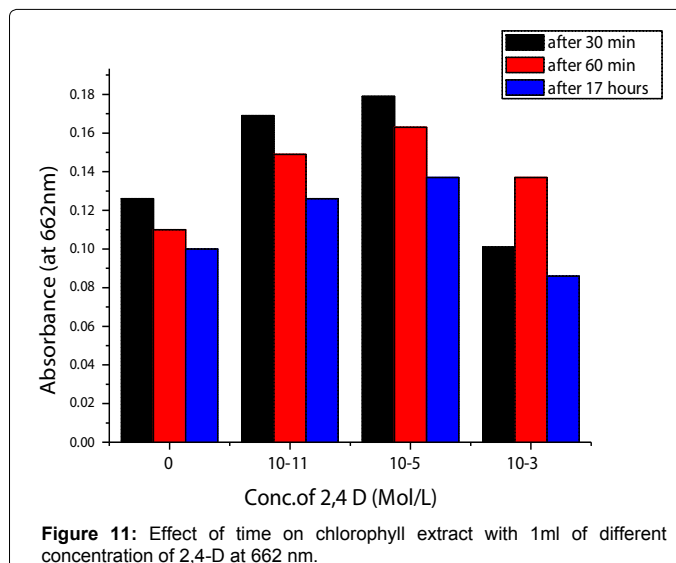
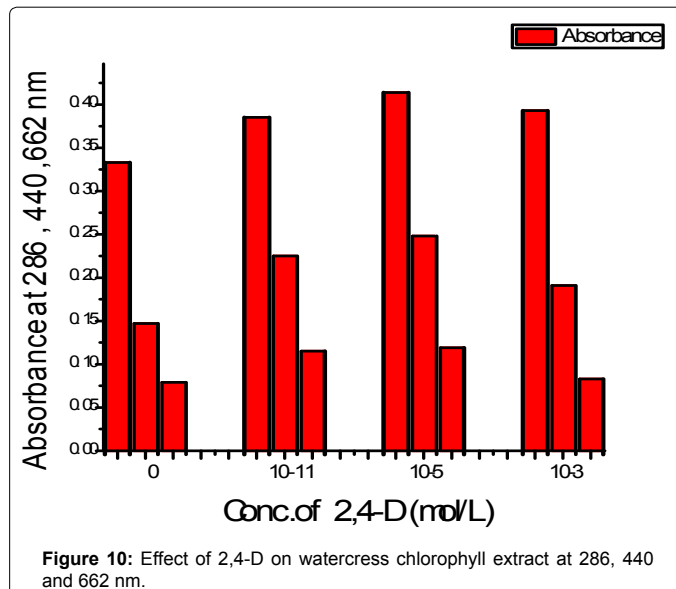
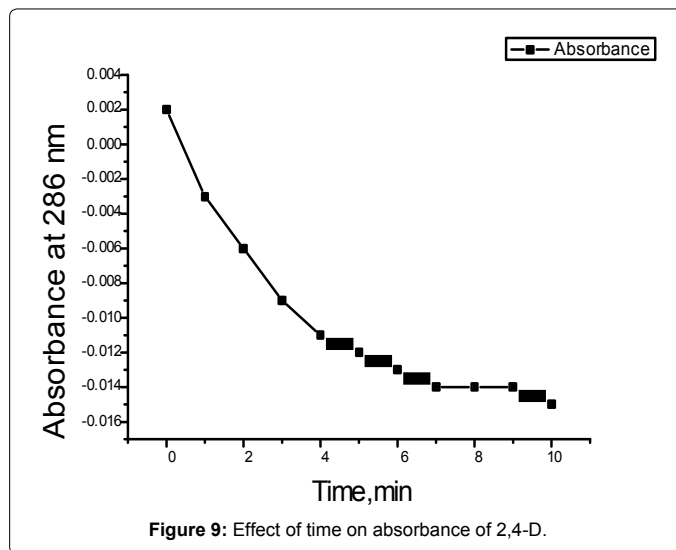
2,4-D concentration is one of the affecting factor to the rate of reaction, so that affects to the performance of silicate ion selective electrode. It has been found that separate technique is the best way to apply Nernst equation. Response characteristics of the silicate ion selective electrode appear in Table 1, the results showed that the highest sensitivity is 42.1 ng/ml.

The purpose of this study was to investigate the working electrode conditions to obtain the maximum performance. The research studied of the electrode character was pH and response time. The pH optimum of 2,4-D at a range from 2.5 to 5.5. Response time is a measure of the rate of degradation reaction of 2,4-D. The response time with concentration higher than 10^{-4} M were of the order of 15 seconds. The silicate electrode has good selectivity to 2,4-D, in the presence of double-charged metal ions, as Fe^{+2} and Glutamic acid, and has poor sensitivity in the presence of Na_2HPO_4 .

The residual pesticide was decreases to 1.005, 0.663, 0.221 $\mu g ml^{-1}$, after two, three, and five days respectively from the original solution ($2.21 \mu g ml^{-1}$) added on the pots of watercress. The number of plants did not effect on the residual of the pesticide concentration. The residual of 2,4-D degradation of ($10.6 \mu g ml^{-1}$) by the temperature effect was varied from -5 to 43°C (Figure 5), the large amount of residual of 2,4-D was at 43°C, for a period of time following treatment. It is therefore to be expected that temperature may influence the rate of degradation response and govern to some extent, Plants that put out the pesticide at low temperature longer than the age of the plants that put them at a temperature of exterminator other heat. This research study reports the concentrations of residual 2,4-D (pH 2.5-9) taken up by watercress grown. This study showed that the pH 4 of 2,4-D solution is the lower pesticide absorption by soil and watercress plants [16,17].

It is very important to see the effect of different solvent as methanol, ethanol, 1,2-dichloroethane and 25% ethanol on the photodegradation of 10^{-11} M of 2,4-D (Figure 1). It is observed that the degradation of 2,4-D in the ethanol was the maximum and the degradation in methanol was the minimum. The velocity of photodegradation of 10^{-3} M of 2,4-D in 25% ethanol is very high (Figure 3).

2,4-D was studied in the concentration range 10^{-11} : 10^{-2} M . The absorbance was decreased from 10^{-11} : 10^{-5} M the reason is the photodegradation of 2,4-D in this region. Microbial degradation is considered and breakdown of 2,4-D . The most important mechanism of microbial degradation involves the removal of the acetic acid side



Parameter	Value*
Slope, (mV/decade)	56.2 ± 2
Intercep , mV	260 ± 5
Correlation coefficient between mV and -log[H ⁺], (r)	-0.014
Detection limit , M	8.3 × 10 ⁻⁷
Response time for 10 ⁻⁴ M solution,s	15
Working pH range	2.5-5.5
Standard Deviation (SD)	2.61
Relative Standard Deviation(RSD)	4.48

*Average of five replicate

Table 1: Response characteristics of the silicate wire ion selective electrode.

chain. This is followed by ring cleavage and degradation to produce aliphatic acids such as succinic acid. The rate of microbial degradation is dependent upon the water potential ψ , the absorbance increased and obeyed Beer's law from 10^{-5} to 10^{-2} M. The molar absorptivity coefficient equal to $1000 \text{ l mol}^{-1} \text{ cm}^{-1}$. Pigment content of chlorophyll were increased after adding, the reason is photodegradation of 2,4-D in this region. $10^{-3} \text{ M} < 10^{-5} \text{ M} < 10^{-11} \text{ M}$ of 2,4-D (Figure 10) at 286,440 and 662 nm. The absorption decreasing with the effect of time (Figure 11).

Conclusion

Silicate wire electrodes was suitable for the determination of 2,4-D with regard to working concentration range, slope, pH range, response time, and selectivity. The electrodes exhibited good reproducibility over a useful lifetime of 2 months. Spectrophotometric study allows understand 2,4-D toxicity and its excellent deleterious effect. The reproducibility, recovery, and operational simplicity of the methods make them suitable to determined 2,4-D. The detection limit was $2.21 \mu\text{g ml}^{-1}$.

Acknowledgements

The author would like to thank the publishers for their interest in reviewing find.

References

1. WHO (2013) The WHO recommended classification of pesticides by hazard.
2. Maleki N, Safavi A, Shahbaazi HR (2005) Electrochemical determination of 2,4-D at a mercury electrode. *Analytica Chimica Acta* 530: 69-74.
3. Gaultier J, Farenhorst A, Cathcart J, Goddard T (2008) Degradation of [carboxyl-¹⁴C] 2,4-D and ring-U-¹⁴C] 2,4-D in 114 agricultural soils as affected by soil organic carbon content. *Soil Biol Biochem* 40: 217-227.
4. Halter MT (1980) 2,4-D in the Aquatic Environment: Section II in Literature Reviews of Four Selected Herbicides: 2,4-D, Dichlobenil, Diquat, and Endothal. Municipality of Metropolitan Seattle
5. World Health Organization (1984) 2,4-Dichlorophenoxyacetic acid (2,4-D). Environmental Health Criteria 29. International Programme on Chemical Safety, Geneva.
6. Frank R, Logan L, Clegg BS (1991) Pesticide and polychlorinated biphenyl residues in waters at the mouth of the Grand, Saugeen, and Thames Rivers, Ontario, Canada, 1986-1990. *Arch Environ Contam Toxicol* 21: 585-595.
7. Shane S, Que Hee, Ronald G, Sutherland (1981) The phenoxyalkanoic herbicides: Chemistry, analysis, and environmental pollution. Chemical Rubber Company Series in Pesticide Chemistry. CRC Press, Boca Raton, USA.
8. Tucker SP, Reynolds JM, Wickman DC, Hines CJ, Perkins JB (2001) Development of sampling and analytical methods for concerted determination of commonly used chloroacetanilide, chlorotriazine, and 2,4-D herbicides in hand-wash, dermal-patch, and air samples. *Appl Occup Environ Hyg* 16: 698-707.
9. Cook LW, Zach FW, Klosterman HJ, Bristol DW (1983) Comparison of free and total residues of (2,4-dichlorophenoxy)acetic acid and 2,4-dichlorophenol in millet resulting from postemergence and preharvest treatment. *J Agric Food Chem* 31: 268-271.
10. Williams KJ, James CR, Thorpe SA, Reynolds SL (1997) Two analytical methods for the measurement of 2,4-D in oranges: An ELISA screening procedure and a GC-MS confirmatory procedure. *Pesticide Science* 50: 135-140.
11. Kashyap SM, Pandya GH, Kondawar VK, Gabhane SS (2005) Rapid analysis of 2,4-D in soil samples by modified Soxhlet apparatus using HPLC with UV detection. *J Chromatogr Sci* 43: 81-86.
12. Park JY, Choi JH, Abd El-Aty AM, Kim BM, Park JH, et al. (2011) Development and validation of an analytical method for determination of endocrine disruptor, 2,4-D, in paddy field water. *Biomed Chromatogr* 25: 1018-1024.
13. Jan MR, Shah J, Bashir N (2006) Flow injection spectrophotometric determination of 2,4-D herbicide *Journal of the Chinese Chemical Society* 53: 845-850.
14. Mostafa GA, Hefnawy MM, Al-Majed A (2007) *Sensors* 7: 3272-3286.
15. IRAC (1987) Overall evaluations of carcinogenicity: an updating of IARC Monographs volumes 1 to 42. IARC Monogr Eval Carcinog Risks Hum Suppl 7: 1-440.
16. Zetterberg G, Busk L, Elovson R, Starec-Nordenhammar I, Rytman H (1977) The influence of pH on the effects of 2,4-D (2,4-dichlorophenoxyacetic acid, Na salt) on *Saccharomyces cerevisiae* and *Salmonella typhimurium*. *Mutat Res* 42: 3-17.
17. Ghassemi M, Fargo L, Painter P, Quinlivan S, Scofield R, et al. (1981) Environmental Fates and Impacts of Major Forest Use Pesticides, Office of Pesticides and Toxic Substances, Washington DC, USA.