



An Ultra-Specific Chromogenic Reagent for TLC Detection and Identification of Paraquat and Design of an Ultra-Low-Cost Sensor for the Field Detection of Viologens

**Asif M. Pathan¹, Mohammad A. Baseer², Archana B. Kadam²
and Subhash B. Junne^{2*}**

1. Regional Forensic Science Laboratory, Nanded, Maharashtra, **INDIA**

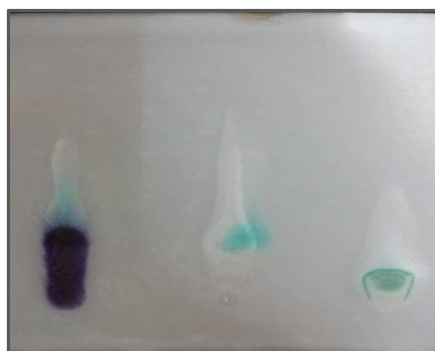
2. Post Graduate Department of Chemistry, Yeshwant Mahavidyalaya, Nanded, Maharashtra, **INDIA**
Email: chemdyesh@gmail.com

Accepted on 30th November, 2018

ABSTRACT

A novel chromogenic spray reagent is reported for thin-layer chromatographic detection and identification of widely used but highly toxic herbicide paraquat. Paraquat is instantly reduced by alkaline stannous chloride to an intense purple compound which is a radical cation of the parent compound. The reagent is suitable for spot test, wet test and also as a thin-layer chromatographic staining reagent with approximate sensitivity of 10 µg. As the reagent is ultra-specific for viologens, a low-cost chalk based chemosensor is also designed for instant on the field detection of viologens.

Graphical Abstract



TLC showing reduction of paraquat by stannous chloride to purple compound.

Highlights:

- A novel thin-layer chromatographic staining reagent for detection of paraquat herbicide.
- Ultra-low-cost chalk based sensor for on the field forensic investigation of paraquat.

Keywords: Paraquat Herbicide, Viologens, Chromogenic spray reagent, Stannous chloride, Chalk based sensor, Chemosensor for viologens.

INTRODUCTION

The use of herbicide in agriculture is necessary to prevent growth of herbs. The herbicide kills herbs and is accepted as useful agrochemicals along with pesticides. The ever growing world's population demands more food and concept of "food security" is the prime human need in this time. While organic foods are in demand by health cautious consumers, the price of organic food materials is high enough in most cases and is not affordable to everyone. The organic pesticides are found to be useful and much less harmful but the overall concept of organic farming has not grown to the pace as compared to traditional chemical farming as of now. The use of synthetic chemical compounds is thus widely adopted. While the agrochemicals are useful to improve production, many of them had been proved to be lethal to human. Many cases of homicidal or accidental and suicidal poisoning are reported due to ingestion of these compounds. Paraquat is one such herbicide which is highly poisonous to human. The LD^{50} value of paraquat for rat was found to be only 100 mg Kg^{-1} [1]. The easy and unmonitored availability of this herbicide has also caused many human poisoning cases [2]. The commercial form of Paraquat and diquat is green colored due to added dye for its differentiation from beverages. However same is the case with Quinalphos (Ekalux) and Dichlorvos (Nuvan). Due to similar appearance of these poisons, an autopsy surgeon cannot give definite opinion on which poison is involved in such medico legal cases. Hence such cases need deeper chemical analysis. Many methods are reported for detection of paraquat which include instrumental methods like gas chromatographic method [3, 4] HPLC method [5, 6], spectrophotometric method [7, 8], voltametric method [9] and thin-layer chromatographic methods [10]. However thin-layer chromatographic methods using direct detection with chromogenic reagent and without the help of any instrument don't seem to be reported. As a primary screening technique TLC is popular but thin-layer chromatographic separation of paraquat gives separation of green dye in it. The detection of herbicide itself requires a staining reagent. Unfortunately very few staining reagents are reported for detection and identification of Paraquat and that too are designed for spectrophotometric detection and lack versatility. Ascorbic acid [8] reacts slowly. It is wellknown phenomenon that a fairly concentrated solution of paraquat changes its colour to blue or purple on standing. A reagent which acts slowly retain doubts in analysis as conclusion cannot be drawn whether the colour change is due to auto reduction or due to added reagent. A blank test is always necessary in such cases. The reagent like glucose [11] can reduce the paraquat but the reagent itself is susceptible to many reactions of carbohydrates which may limit its usage for complex matrices like viscera. Sodium dithionite [12] reduces paraquat instantly but its preparation require sulphur dioxide which is harmful to environment. Sodium borohydride [13] and related hydride donor reducing agent may be difficult to handle due to their tendency to produce highly flammable hydrogen gas. Due to all these difficulties there was need of a chromogenic reagent which is not prone to side reactions, versatile, very fast in action and safe to handle. This manuscript describes a novel colour reaction of paraquat with stannous chloride. The reaction is further applied as chromogenic reagent for detection and identification of paraquat and also for designing a novel chalk based sensor which can be directly used on the fields and crime scenes.

MATERIALS AND METHODS

Apparatus and Chemicals: Stannous chloride (mfg by Tirupati industries (India) Ltd., Raigad, Maharashtra, India), Sodium hydroxide, Paraquat dichloride (as 24% solution, brand name Paragreen, mfg by Greencrop international, Pune, Maharashtra, India), Dichlorvos (as 79% solution, brand name DDVP, brand name, National Pesticides and Chemicals, Mumbai, India), Quinalphos (as 25% solution, brand name Haiquin, mfg by Hindustan Agrotech Industries, Ahmedabad, India), methanol, Ammonia, HPTLC plates (size 10 cm by 10 cm manufactured by Merck), a white chalk for sensor.

Thin-Layer Chromatography: HPTLC plate was directly used for spotting. Paraquat, Dichlorvos and Quinalphos was spotted from left to right respectively. The solvent system was prepared in a glass chamber by mixing 49 mL of Methanol and 1 mL Ammonia. The ascending one dimensional

chromatogram was then run to the desired height and the plate was then removed. The plate was allowed to dry for few minutes until it was free from all mobile phases. Developed TLC shows separation of green dyes in all three compounds. This TLC plate was photographed (Fig 1).

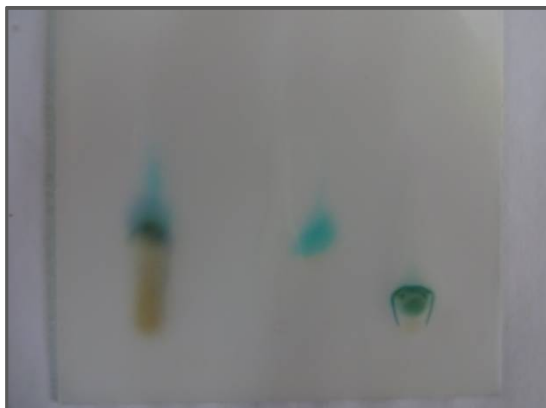


Figure 1 TLC showing separation of green dyes in Paraquat, Dichlorvos and Quinalphos (from left to right).

When the plate was dry, 2% stannous chloride spray solution was prepared by mixing 1 gram of stannous chloride in 50 mL of 2N sodium hydroxide. The precipitate was not removed. The plate was immediately sprayed with this freshly prepared alkaline stannous chloride solution. The post staining TLC plate was also photographed.

RESULTS AND DISCUSSION

All three compounds look similar at the time of spotting, so they can't be distinguished from each other by mere observing them. The pre stained TLC shows separation of green dyes in all three analyte. It is also difficult to distinguish them by merely observing their running pattern. After spraying the plate with alkaline stannous chloride, only paraquat spot is converted to bright purple compound which is radical cation of the original compound (Fig 2).

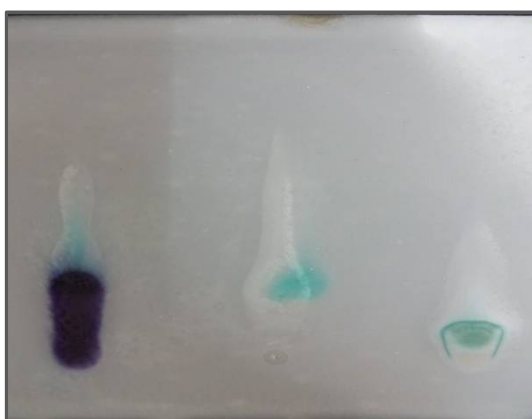


Figure 2. TLC showing reduction of paraquat by stannous chloride to purple compound.

Paraquat and diquat belongs to a family of redox-active compounds called as viologens. Viologens are reduced by various reducing agents to blue or purple radical cation, the chemistry of this colour reaction is well established [14].

The probable reaction is shown in figure 3.

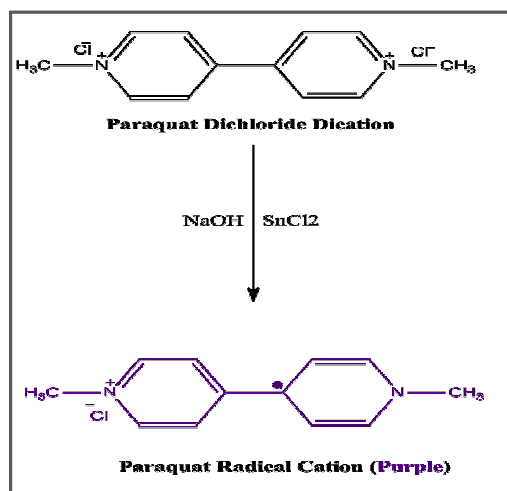


Figure 3. Probable mechanism of reduction of paraquat by alkaline stannous chloride.

It is known that stannous chloride acts as reducing agent in strong acidic medium. The reagent is used specifically to reduce aromatic nitro group to amino group. However acidic medium is prime requirement for this reduction. Acidic stannous chloride however, does not reduce paraquat at all. Hence alkaline stannous chloride acts completely different from acidic stannous chloride. The other organophosphorus insecticides like rogor, malathion, thimet, organochloro insecticides like DDT, BHC, endosulfan, carbamate insecticides like carbaryl, carbofuran, carbosulfan, pyrethroid insecticides like cypermethrin, deltamethrin and λ -cyhalothrin doesn't show any coloured product. Thus the reagent is very specific for paraquat and diquat.

APPLICATION

Design of an ultra-low-cost sensor: The routine forensic investigation of suicidal, homicidal or accidental poisoning involves a post-mortem of victim's body and laboratory analysis of viscera. The non-biological exhibits seized from crime scene are also sent to laboratory for poison detection. The process of collecting non-biological exhibits from crime-scenes and sending them further to forensic laboratory has very strong inherent limitations. First of all it requires an expert's help to collect some tedious samples. Some samples may have time bound activity. There is a waste of time, money and manpower in collecting each and every sample from crime scene and further forwarding them to laboratory. The critical weather conditions can multiply these teething troubles. All these problems can be solved if at least some samples can be checked at the crime scene itself. To do this, a miniature version of lab test-kit is needed to be created. The desirable feature of such miniature test kit is its portability, reliability, speed and accuracy. A chemosensor can satisfy all these conditions [15]. Due to their portable nature they can be carried to the field. They can be used in extreme weather conditions. They can give valuable, quick and reliable results even before the lab tests and certainly improve natural justice system by speeding up the investigations.

Based on reported novel chromogenic reaction, we developed, tested and successfully applied the sensor to detect presence of paraquat on few forensic samples. The sensor was prepared by taking a regular white chalk and dipping the chalk to half of its height into alkaline stannous chloride solution. To detect the presence of paraquat in liquid samples, the sensor was dipped in that liquid. An instantaneous bright purple colour develops at the dipped end. The presence of paraquat can also be confirmed on solid sample such as a piece of cloth by rubbing the chalk on that solid surface all the way and adding few drops of sodium hydroxide on it. The sensor can be carried at the field and should include additional sodium hydroxide in separate bottle to be used for solid matrix. Figure 4 shows working of sensor with liquid matrix as well as solid matrix.

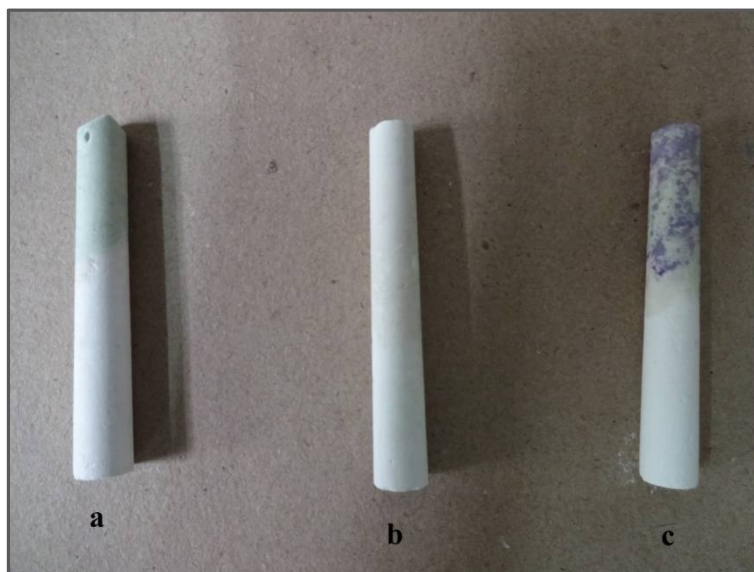


Figure 4. The working of chalk-based sensor: a). contains only paraquat, b). contains only alkaline stannous chloride and c). contains paraquat spotted over alkaline stannous chloride.

CONCLUSION

A novel staining reagent is reported for thin-layer chromatographic detection and identification of very toxic herbicide paraquat. The reported method not only identifies paraquat but also easily distinguish it from dichlorvos and quinalphos. The work probably reports the first method to distinguish these very similarly looking herbicide and pesticides. The reagent is specific, sensitive and cheap. The staining reagent so reported is versatile and can be used for wet test, spot test as well as regular staining reagent for TLC. The designed sensor can prove precious in forensic investigations. The chalk-sensor can even be utilized by medical professionals to detect the paraquat in stomach wash or vomit samples which initially appear green in colour. The early detection of poison on the field and in hospitals can even save countless lives by giving correct antidote. Conversely, the paraquat in alkaline condition can also be used to detect presence of stannous (Sn^{2+}) ions, thus working as an 'organic analytical reagent' for stannous. A role of very dilute stannous chloride as an antidote for paraquat poisoning should also be tested and may open up new ways for clinical research in this direction.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. A.N Jadhav, Principal, Yeshwant Mahavidyalaya, Nanded for providing the necessary laboratory facilities and to Dr. K.V. Kulkarni, Director, Directorate of Forensic Science Laboratories, Mumbai for his helpful suggestions during this work.

REFERENCES

- [1]. R. D. Kimbrough, T. B. Gaines, Toxicity of paraquat to rats and its effect on rat lungs, *Toxicology and applied pharmacology*, **1970**, 17(3), 679-690.
- [2]. C. M. Bullivant, Accidental poisoning by paraquat: Report of two cases in man, *British Medical Journal*, **1966**, 1(5498), 1272.
- [3]. S. U. Khan, Determination of paraquat residues in food crops by gas chromatography. *Bulletin of environmental contamination and toxicology*, **1975**, 14(6), 745-749.

-
- [4]. Kawase, Shōzi, Susumu Kanno, Shigeo Ukai. Determination of the herbicides paraquat and diquat in blood and urine by gas chromatography, *Journal of Chromatography A*, 1984, 283, 231-240.
- [5]. P.Paixao, Simple method for determination of paraquat in plasma and serum of human patients by high-performance liquid chromatography, *Journal of chromatography B*, 2002, 775(1), 109-113.
- [6]. Corasaniti, Maria Tiziana, Giuseppe Nisticò, Determination of paraquat in rat brain by high-performance liquid chromatography, *Journal of Chromatography A*, 1993, 643(1-2), 419-425.
- [7]. S. H. Yuen, J. E. Bagness, D. Myle., Spectrophotometric determination of diquat and paraquat in aqueous herbicide formulations, *Analyst*, **1967**, 92(1095), 375-381.
- [8]. Shivhare, Priti, V. K. Gupta, Spectrophotometric method for the determination of paraquat in water, grain and plant materials, *Analyst*, **1991**, 16(4), 391-393.
- [9]. Qiu, Ping, Yongnian Ni, Serge Kokot, Simultaneous voltammetric determination of four triazine herbicides in water samples with the aid of chemometrics, *Journal of Environmental Science and Health, Part B*, **2014**, 49(10), 722-729.
- [10]. Ikebuchi, Jun, Isao Yuasa, Susumu Kotoku, A rapid and sensitive method for the determination of paraquat in plasma and urine by thin-layer chromatography with flame ionization detection, *Journal of analytical toxicology*, **1988**, 12(2), 80-83.
- [11]. R. Khatoon, M. K. Rai, J. K. Rai, Low cost spectro photometric determination of paraquat in environmental and biological sample, *Recent Research in Science and Technology*, **2013**, 5(2), 4-6.
- [12]. A. Calderbank, S. H. Yuen, An ion-exchange method for determining paraquat residues in food crops, *Analyst*, **1965**, 90(1067), 99-106.
- [13]. M. K. Rai, Joyce Vanisha Das, V. K. Gupta, A sensitive determination of paraquat by spectrophotometry, *Talanta*, **1997**, 45.2, 343-348.
- [14]. L. Michaelis, Edgar S. Hill, The viologen indicators, *The Journal of general physiology*, **1933**, 16(6) 859.
- [15]. R. Muller, Chemical sensing and pattern recognition, *COMPEURO 89 Proceedings VLSI and Computer Peripherals*. IEEE, **1989**.