THE UNUSUAL BEHAVIOR OF GIBBS' REAGENT VERSUS NITROFURAL

NARASAIAH MUPPURI
Dept of Chemistry,
SKBR Government Degree College, Macherla-522426, AP, India.
E. Mail: narasaiah81@gmail.com

ABSTRACT

A novel optical sensor for the rapid and direct determination of permethrin preservatives in treated wood was designed. The optical sensor was fabricated from the immobilization of 2,6-dichloro-p-benzoquinone-4-chloroimide (Gibbs reagent) in nafion/sol–gel hybrid film and the mode of detection was based on absorption spectrophotometry. Physical entrapment was employed as a method of immobilization.

Keywords: *2,6-dichloro-p-benzoquinone-4-chloroimide; Permethrin; Nafion; Sol–gel; Optical sensors*

INTRODUCTION

Preservatives have been widely used in wood preservation process, agriculture, chemical, and polymer technology to protect various products against decay by biodegradation. The choice of preservative to protect a product such as wood-based materials and vegetables is based on the chemical properties of the preservative. Wood preservative usually consists of a mixture of preservatives. Wood preservative acts as an antifungal agent and insect repellent. In general, a preservative must have an appropriate level of toxicity to prevent spoilage from molds and to prevent insects from attacking wood or vegetable.

In the past, preservatives such as lindane, dieldrin, aldrin, and chlorpyrifos were widely used. Nowadays, these chemicals are largely replaced with pyrethroid group of preservatives such as permethrin and cypermethrin. Permethrin is often used to protect wood from termite attack. The advantage of using this insecticide is that it is active in small doses and has a low toxicity to humans. Therefore, permethrin is used in solvent-based systems for the treatment of wood-based composites.

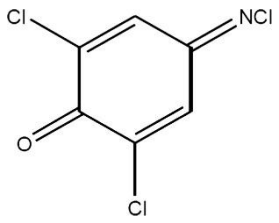


Figure Structure of 2,6-dichloro-p-benzoquinone-4-chloroimide reagent.

Usually, the quality of permethrin treatment in wood and vegetables is analysed using gas chromatography (GC), liquid chromatography (LC), immunology, and electronic nose. These instrumental methods can normally determine permethrin concentrations in wood or vegetables according to the specifications set by standard procedures associated with the effective prevention of pest attack that causes biodegradation. Gas chromatography (GC) and liquid chromatography (LC) are techniques that cannot be used for *in situ* determination of permethrin. In addition, the sample for both techniques also requires an extraction step, which very often is time consuming. Other than that, the use of electronic noses could only detect permethrin qualitatively,

i.e., whether it is present or absent in a sample.

In this study, an optical chemical sensor for the detection of permethrin in treated wood was developed. The new chemical sensor concept was based on the reaction between permethrin and 2,6-dichloro-p-benzoquinone-4-chloroimide reagent (Gibbs reagent). Gibbs method is a standard method used for the detection of phenol. The method is based on the condensation reaction between dichloroquinone-4-chloroimide with phenol compounds that do not have a successor group to form a compound of the 2,6-dichlorophenol. The reaction takes place in an alkaline medium at pH 9.4 of borate buffer. For the determination of phenol in the range of ppm, 2,6-dichlorophenol compounds give absorption at a wavelength of 595 to 630 nm.

In addition, such factors as temperature, pH, and presence of other compounds such as sulphide, reducing agent, and thiocresol have been found to affect the reaction. The structure of Gibbs reagent is shown in Figure 1. Until now, there is report regarding the reaction between permethrin and Gibbs reagent. An optical sensor is fabricated to detect permethrin by using 2,6-dichloro-p-benzoquinone-4-chloroimide reagent immobilised in a nafion and sol–gel silicate hybrid membrane. In this study, the performance of the chemical sensor for the analysis of permethrin in treated wood was validated with standard methods.

LITERATURE REVIEW

Pratt WB; Scholar EM. (2013) A precise, simple, cost-effective, specific and sensitive spectrophotometric method was developed for the assay of Dopamine HCl. The proposed method is based on generation of a colored complex through utilizing the known reaction of 2,6-dichloroquinone 4-chloroimide (DCQ) with phenols, primary and secondary amines. Dopamine HCl reacts with 2,6-dichloroquinone 4-chloroimide (0.12% solution in ethanol), in aqueous media at room temperature to produce a colored product that absorbs light at λmax 470 nm. All reaction conditions were optimized and standardized.

Pedersen O. (2006) The absorbance intensity of the colored product was linear with Dopamine HCl concentration in the range of (5 to 45 µg/ml). The correlation coefficient was found to be (r =0.999). The limit of detection was 2.5µg/ml. The stoichiometry of the reaction between Dopamine HCl and 2,6-dichloroquinone 4-chloroimide was studied, and revealed a 1:4 ratio of Dopamine HCl: DCQ respectively. The added recovery and standard addition approaches' results were 99.67%±2.25 (n=6) and 99.09% for the former and later respectively, indicating the absence of interference.

Weygand C, Hilgetag G (2012) The study covers a new qualitative analytic test (method) for detecting the semicarbazone chemotherapeutic Nitrofural. The combination of Gibbs' reagent and ammonia was successfully implemented for analyzing the drug. The main structural features of the obtained color products were determined by UV-VIS spectroscopy

Kramer DN, Gamson RM. Preparation of quinone sulfenimines. J Org Chem (2019)A simple, sensitive, cost-effective, and accurate, spectrophotometric method is described for the assay of Dopamine HCl in bulk and injectable form. The

method is based on the generation of a brownish-orange colored product that absorbs at λmax 470 nm through the reaction of Dopamine HCl with DCQ in aqueous media at room temperature. The simplicity and cost-effectiveness of the developed method make it suitable for routine quality control analysis of Dopamine HCl especially for colored coated tablets.

Gibbs HD. (2000) The study covers a new qualitative analytic test (method) for detecting the semicarbazone chemotherapeutic Nitrofural . The combination of Gibbs` reagent and ammonia was successfully implemented for analyzing the drug. The main structural features of the obtained color products were determined by UV-VIS spectroscopy.

Feigl F, Jungreis E, Oesper RE. (2009) The sensor was useful for rapid screening of wood or treated wood products before detailed analysis using tedious procedure is performed. The validation study of the optical sensor against standard method HPLC successfully showed that the permethrin sensor tended to overestimate the permethrin concentration determined.

METHODOLOGY

Reagents

Dopamine HCl working standard (assigned purity 99.83%), was supplied by the Central Medicines Supply (C.M.S-Sudan). Distilled water is used all through, together with chemicals & reagents of analytical grade. Phosphate buffers of pH 3 and 7, and borate buffer were prepared according to the B.P.2010 procedure. DCQ reagent was freshly prepared by dissolving appropriate amount in absolute ethanol to prepare 0.1, 0.12, 0.24 % w/v solutions.

Pharmaceutical Formulation

Sterile Dopamine HCl concentrate BP [each ml contains Dopamine HCl BP 40 mg, water for injection BP q.s], manufactured by Claris Lifesciences Limited, Batch No. A080473, Mfg. date 07-2008, Exp. Date 06-2010, India.

Preparation of Standard Solution:

A weight of 0.0125 g of Dopamine HCl RS were dissolved in distilled water, transferred into 50 ml volumetric flasks, diluted to volume and mixed. Serial dilutions were made to obtain 0.18 mg/ml, 0.1 mg/ml and 0.09mg/ml working solutions.

Experimental

All absorbance readings were measured using UV-1800 Shimadzu Spectrophotometer connected to hp Laserjet 1300 printer or Perkin Elmer UV/VIS Spectrometer lambda 2 connected to Perkin Elmer Ex-800 printer.

Standard Calibration Curves

To 0.5, 1.0, 1.5, 2.0, and 2.5 ml of the Dopamine HCl (0.18 mg/ml working solution), 2.0, 1.5, 1.0, 0.5, 0.0 ml of distilled water was added respectively. One ml of DCQ (0.12 %) was added, mixed, and reaction mixture allowed standing for 60 minutes. The volume was then completed to 10 ml using distilled water. Absorbance was measured at 470 nm against reagent blank prepared similarly. Calibration curves were constructed by plotting the absorbance readings at 470 nm versus concentrations of Dopamine HCl.

Pharmaceutical Preparation

4.5 ml (180 mg) from injection were pipetted and diluted to 50 ml with distilled water to obtain a solution of 3.6 mg/ml concentration (solution A). Solutions of concentrations of 1.8 mg/ml and 0.18 mg/ml were prepared from solution A through appropriate dilutions. Aliquots of the sample solutions were taken and prepared similarly as for standard calibration curves.

Procedure: Each microtube was loaded with appropriate volumes of solutions A, B, C, D, and E, and then sealed with a cap with a silicon Oring. All tests were repeated three times to confirm the repeatability of the analysis.

RESULTS

The Gibbs' reagent has the ability to bind to various different unhindered phenolic structural motifs, some esters, certain thiols and sulfhydryl groups, nitroxyl groups, amines, and some aliphatic and aromatic acid hydrazides, as well as to aldehyde hydrazones. The Gibbs' reagent has been and is still used as a powerful analytical tool for the qualitative and quantitative analysis of many pharmacopoeial representatives, such as orciprenaline sulfate, Cresolum crudum, vitamins B6 and K, theophylline, methylthiouracil, the anesthetic propofol, as well as of some antibiotics and opiates. The presence both of all aforementioned compounds and many other non-pharmacopoeial analytes can also be verified using the "Gibbs' spray" (TLC developer).

In addition to a liquid medium, the reactivity of Gibbs' reagent has been found to be intact both in the solid state and at the so-called point of solvent evaporation (semisolid state). It is supposed that the mechanism of all foregoing analytical reactions most probably is based on the ability of the Gibbs' reagent to come into direct contact with the active CH (or CH2) functional centers of the tested analytes. As expected, a negative result has been observed in evaporating drops of both reactants4 . An analogous behavior of the tested semicarbazone toward Gibbs' reagent has also been evinced under solid-phase reaction conditions5 . Inertness of the chloroimide reagent in relation to the tested medicine has likewise been registered both in neutral and in a weakly acidic organic medium.

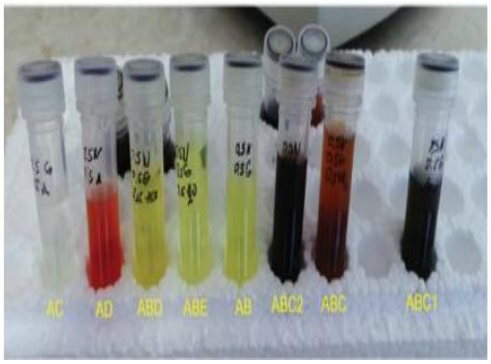
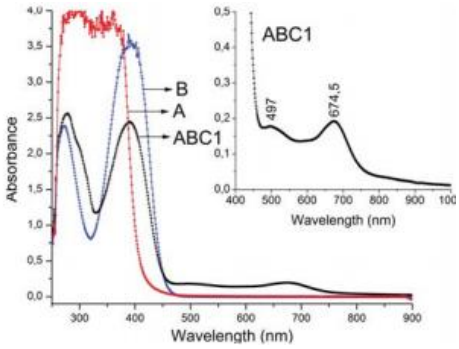


Figure A photograph illustrating the differences in appearance of all reaction products

Surprisingly, however, in weakly ammoniacal milieu a positive qualitative response (revealed by the appearance of a seaweed green colored product) has been obtained. The appearance of a dark seaweed green colored product, very likely, has come as a result of the electrostatic interaction between an easily heterolytically dechalogenated N-haloimide "radical" and the α-aza-stabilized carbanion of the reacting semicarbazone (a carbanion, which can be formed in situ by adding ammonia to the tested weak acidic Nitrofural NH center).

This reaction is presumed to proceed through an unstable N- (diazenylmethyl) methanimine, "intermediate", which tends to isomerize to the more stable and green colored 1-diazenyl-N-methylmethanimine product.

At first glance, the mechanism is somewhat astonishing since ammonia is ordinarily not perceived as a classic example for such a strong proton acceptor. However, its action as a base is comprehensible when keeping in mind the behavior of a weak acid that the tested analyte exhibits. In this case, anhydrous ethanol was used as resolving and easily volatizing agent for the examined analyte. Reaction accomplished by gentle rubbing of both reactants with a glass rod on a glass spot plate. To exclude the presence of artifacts (complications by side reactions), additional tests were performed in the following order: AC, AD, ABD, ABE, and AB.



Graph UV-VIS spectra of the Gibbs reagent (A), Nitrofural (B), and its product of interaction in ammoniacal milieu (ABC1)

As seen from Fig, DMSO solutions of Nitrofural remain practically unaltered upon the addition of the Gibbs' reagent (AB), H2 O, and HCl. The same is valid for their mixtures (ABD, ABE). In the presence of ammonia (in traces), however, a slight coloring of the Nitrofural solution toward the orange gamma (spectrum) occurred within 3 minutes. No color change was observed toward alkalization of the Gibbs' reagent (AC) all along of the analysis. Again, however, an unexpected stratification of the explored analytical mixture (AB) was observed upon adding an excess (≥0.25 ml) of ammonia (cases ABC and ABC2). Withal in the course of analysis, a complex set of color changes in the compositions of the obtained phases were also registered. It is safe to say, a posteriori, that the observed analytical changes (in color and phase) in the two final trials were too involved and complicated to be evaluated analytically and systematically with the required analytical quality.

UV-Vis Analysis

The 1-diazenyl-N-methylmethanimine product formation was confirmed by UV-VIS spectroscopy . As seen from Fig. , the UV-Vis spectra of all investigated samples have a similar absorption pattern in the short wavelength (UV) range (from 250 to 330 nm). A slight deviation was only registered in the position of the absorption extremum, as well as in the profile (reflected by the appearance of an absorption shoulder at the direction of the red region) of this high energy absorption band in the spectrum of the reaction product (ABC1). The observed spectral effect may be ascribed to the presence of overlapping absorption peaks of the corresponding unreacted reaction species (A and B) – admissible presence of reactants in the composition of the reaction product; it should not be forgotten that the characteristic features of this spot test are manifested toward an insignificant amount of the applied reactants (as it was pointed out supra in traces of ammonia).

As regards the absorption peak with a maximum at 440 nm in the spectrum of the green colored diazenylmethanimine product, no significant deviations from the spectrum of the used Nitrofural substrate (B) have been observed. The successful inclusion of a chromogenic element into the Nitrofural backbone has been unambiguously proven by the presence of 2 well-defined peaks (with clear and definite shape and maxima) at about 497 and 675 nm in the spectrum of the formed product. It is suggested that the appearance of these two bands in the spectrum of the compound in question should, most probably, be attributed to the presence of an analyte-specific, extensively conjugated chromophoric system, composed by a compactly arranged (delocalized) double bonds, which are capable of interacting with the electromagnetic radiation in the visible spectral range.

CONCLUSION

A new, two-step colorimetric test for qualitative determination of Nitrofural using the Gibbs reagent as a chromogenic coupling agent has been successfully developed. In a series of tests, the proposed colorimetric test has proven its reliability and efficiency in the qualitative analysis of the medicine in question. The colorimetric test differs from the existing pharmacopoeial approach in being completely defined by a set of experimental circumstances (factors). The presented method may also be used as experimental proof revealing the hidden possibility of interaction of semicarbazones with the Gibbs reagent. Furthermore, the method employs inexpensive and easily available chemicals. The workflow of the presented procedure is simple, rapid, reproducible, and employable with standard laboratory equipment. It can also be accomplished within the range of the so-called "expressive spot test analysis" by a wide range of investigators even with less analytical experience.

REFERENCES

1. Pratt WB; Scholar EM. The antimicrobial drugs. New York: Oxford University Press; 2000.

2. European Pharmacopoeia, Strasbourg: Council of Europe. 8th ed., vol. 1 and 2;2013.

3. Pedersen O. Pharmaceutical Chemical Analysis: Methods for identification and limit tests. Boca Raton: Taylor & Francis Group; 2006.

4. Weygand C, Hilgetag G (ed. by Hilgetag G. and Martini A.). Preparative Organic Chemistry. John Wiley & Sons; 2012.

5. Gibbs HD. Phenol tests III. The indophenol test. J Biol Chem. 2000;72(2):649-64.

6. Feigl F, Jungreis E, Oesper RE. Spot tests for phenol vapors and for aromatic compounds containing oxygen. Anal Chem. 2009;31(12):2099-101. doi: 10.1021/ac60156a070.

7. Josephy DP, Damme AV. Reaction of Gibbs reagent with para-substituted phenols. Anal Chem. 1984;56(4):813–14. doi: 10.1021/ac00268a052.

8. Pallagi I, Toró A, Horváth G. Mechanism of the Gibbs Reaction. Part 4. (1) Indophenol formation via n-chlorobenzoquinone imine radical anions. The Aza-S(RN)2 chain reaction mechanism. Chain initiation with 1,4-benzoquinones and cyanide ion. J Org Chem. 1999;64(18): 6530–40.

9. Kramer DN, Gamson RM. Preparation of quinone sulfenimines. J Org Chem. 2019;24(8):1154–5. doi: 10.1021/jo01090a623.

10. Gamson RM, Kramer DN, Miller FM. A study of the physical and chemical properties of the esters of indophenols. II. Structural studies of the isomeric esters. J Org Chem. 1959;24(11):1747–50. doi: 10.1021/jo01093a033.

11. Harfoush AA, Zagloul A, Abdel Halim FM. Kinetics of the reaction of N-2,6-trichlorobenzoquinonimine with sodium

thiosulphate. Monatshefte Für Chemie/Chemical Monthly. 1982;113(6-7):837–43. doi: 10.1007/BF00809024.

12. Harfoush AA. Medium effect on the reaction of Gibb's reagent with sodium thiosulphate. J Chem Technol Biotechnol. 1983;33A(6):281–5. doi: 10.1002/jctb.504330602.

13. De Boer EJM, Heijden HVD, Verhoef-Van Wijk WC, Zon AV. Ligands and catalyst systems thereof for ethylene oligomerisation to linear alpha olefins. United States Patent. 2007;Patent No.:US 7,304,159 B2.

14. Kallmayer HJ, Thierfelder B. Die Reaktion Des Iminodibenzyls Mit 2,6-Dichlor-1,4-Benzochinon4-Chlorimid. Pharmazie 2003;58(3):218–9.

15. Annapurna V, Jyothi G, Nagalakshmi V, Sailaja BBV. Spectrophotometric methods for the assay of fluvoxamine using chromogenic reagents. E-Journal of Chemistry 2010;7(4):1539–45. doi: 10.1155/2010/137178.