

Qualitative and Quantitative Analysis of —OH Substituted Phenols Based on Formation of Complex with Cu(II)

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A method has been developed to assay substituted phenols. It considers that when hydroxyl group is present on an arene ring, it dissociates so as to give negative charge in presence of alkali. In this study different isomers of hydroxyphenols are used. We propose that the addition of divalent metal ion Cu(II) result in the formation of an intermediate metal-arene complex as a precipitate in the assay. The complex formed may have different configuration with respect to the position of hydroxyl group on the phenolic ring. The acid soluble complex imparts colours to the reactions based on their absorption maxima. The spectrophotometric assays have shown the distinct spectra based on the substitution on the phenol ring.

Key words: Qualitative, quantitative, analysis, phenols, Cu(II), complex.

INTRODUCTION

Hydroxysubstituted phenols, *viz.*, catechol, resorcinol, hydroquinone are the contaminants found at various dumpsites. They are also the intermediates or by-products in many chemical syntheses such as dyes and drugs¹. There are recent reports describing the coloured reaction for phenol² and other complex aromatic compounds^{3,4}. However, using the same protocols the hydroxy derivatives of phenol could not be assayed with confidence even at the qualitative level. We are working on characterizing the microbial diversity for various substitute phenol-utilizing bacteria⁵⁻⁷. It involves collection of samples from different dumpsites and on-site analysis for xenobiotic molecules. The qualitative analyses in these kinds of surveys involve close-point characterization of contaminant in acres of land area. This was the motivation for this study. The developed protocol differentiates phenolics on the basis of distinguishing coloured complexes formed. Hence, it could be used as an on-site qualitative analysis tool with limited sensitivity, if extended as a quantitative tool. Initially, three isomers were used in the reactions to form coloured complexes. The method is sensitive at 100 μM concentration. Furthermore, the spectrophotometric identification aids in resolution of the compounds from each other.

EXPERIMENTAL

The visible spectra were recorded using spectrophotometer Perkin-Elmer Lambda 900 model. The stock solutions were made for all the target molecules at 100 mM concentration. Other reagents used in the study were as follows: cupric chloride, 20%; sodium hydroxide, 5 N; nitriloacetic acid, 1%. All solutions were made in double distilled water and all the analytical grade chemicals were purchased from Sigma-Aldrich.

Procedure

Reactions were carried out in 1 mL volume in 1.5 mL tubes. The reaction mixture consisted of 2.5 μL NaOH (5 N), different concentration of target phenolic in 50 μL of double distilled water and 20 μL of CuCl_2 . The final volume was made up to 1 mL by double distilled water. The mixture was shaken vigorously for 10–15 min at room temperature on shaker at 150 rpm. This resulted in the formation of the complex, which precipitated in aqueous medium, which was dissolved by addition of 100 μL of nitriloacetic acid. The reaction was incubated for the next 20 min for intense colour development. The absorbance was read against a blank, which consisted of all the reagents in the same proportion except the target phenolic molecule. By taking different concentrations of cupric ions the method was also optimized.

RESULTS AND DISCUSSION

We describe here a protocol that can assay the hydroxy-substituted phenols with no interference with phenol. The protocol considers the electron sharing property of Cu(II) to react with quinonoid form of phenolics in alkaline medium. The resultant complexes impart colour to the reaction, which can be further intensified on incubation with nitriloacetic acid. The final colour remained stable thereafter for 10 h and with no nitriloacetic acid in the medium colour disappeared in 2 h. Higher concentrations of the target molecule and/or of copper ions resulted in deep intensification of the colour. The spectrophotometric analysis for three

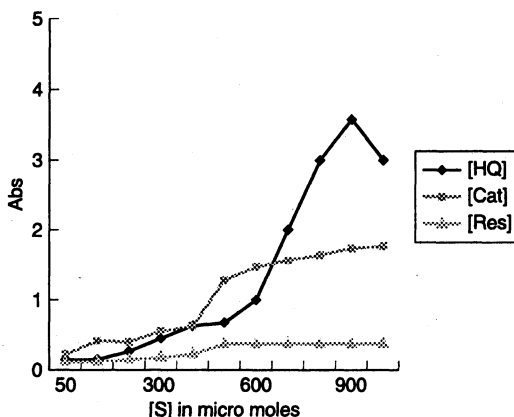


Fig. 1. Absorbances of increasing conc. of the target molecules at their respective wavelengths

different target molecules is shown in Fig. 1; the concentrations used are not beyond 10 mM, though best results are achieved in the range of 500–800 μM of

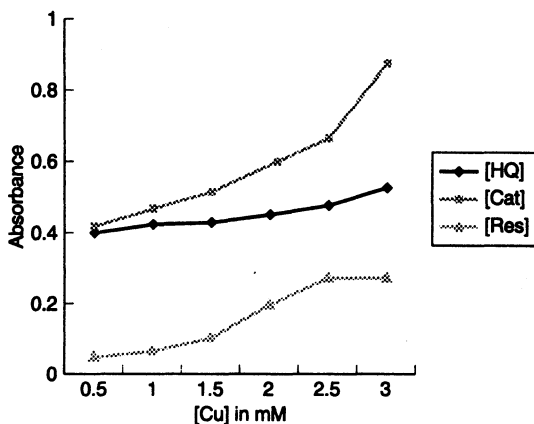


Fig. 2. Absorbances of increasing conc. of Cu(II) at their respective wavelengths (Target molecule concentration used was 300 micromoles in all.)

target molecule concentration. As shown in Fig. 2, the required concentration of Cu(II) for optimum colour development is 2.5 to 3 mM. After 5 mM concentration of Cu(II) the reaction developed turbidity with the used concentrations of phenolics. The reason for increased turbidity could be attributed to the fact that

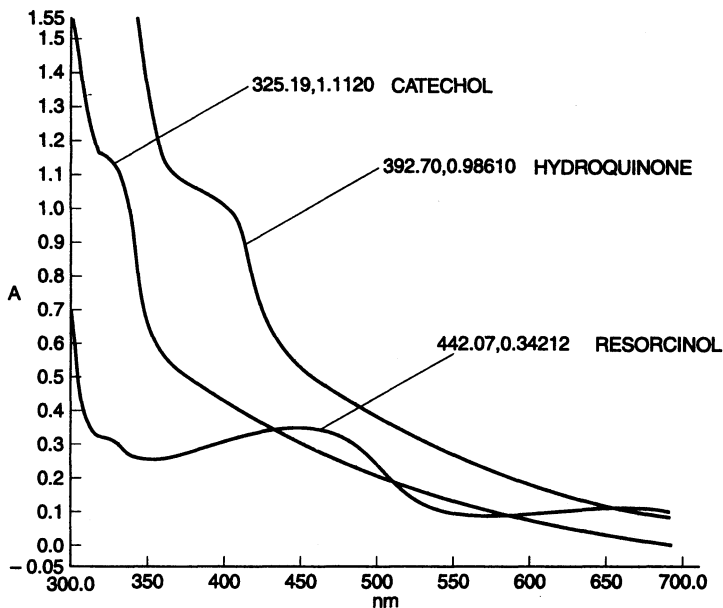


Fig. 3

Cu(II) in presence of NaOH formed cuprous hydroxide. Also, the aromatic molecules used as a target molecule have a tendency to polymerize and precipitate out from the aqueous medium. This phenomenon was more pronounced in the presence of excess of alkali, which gives an envelope of negative charge to the —OH group and hence to the whole aromatic molecule.

Fig. 3 illustrates the absorption maxima of catechol, resorcinol, and hydroquinone each in separate reactions. With the addition of up to 1 mM of phenol in the same reaction there was no change in absorption maxima of all the three target molecules (data not shown). Based on these observations the protocol was extended to -chloro, -nitro, -amino group substituted phenols with reference to *ortho*-, *meta*- and *para*- positions. Table-1 shows the observed colours in the reaction with various target phenolics. There was no colour reaction observed with the chloro group substituted phenol in the reaction. Similarly, with all the nitro substituted phenols only the color intensity of the reaction increased, *i.e.*, more deep colors were observed without change in absorption maxima of the parent molecule.

TABLE 1
CHARACTERISTIC COLOURS AND ABSORPTION MAXIMA
OF —OH SUBSTITUTED PHENOLS

Substrate	Colour of the complex	Absorption maxima
Phenol	—	—
Catechol	Light green	325
Resorcinol	Dark green	442
Hydroquinone	Pink	390
<i>o</i> -amino phenol	Green	448
<i>m</i> -amino phenol	Brown	290
<i>p</i> -amino phenol	Orange	423
Chlorophenols	No colour	—
Nitrophenols	Deep yellow	Around 420
Mixture of phenolics	Dark Green	—

REFERENCES

1. J.C. Spain, *Annu. Rev. Microbiol.*, **49**, 523 (1995).
2. H.-W. Gao, *Asian J. Chem.*, **12**, 511 (2000).
3. S.N. Sharma and P.K. Ambastha, *Oriental J. Chem.*, **16**, 125 (2000).
4. I.A. Mustafa, *Asian J. Chem.*, **13**, 377 (2001).
5. R. Kuty, H.J. Purohit and P. Khanna, *Can. J. Microbiol.*, **46**, 211 (2000).
6. E.I. Atuanya, H.J. Purohit and T. Chakrabarty, *World J. Microbio. & Biotech.*, **16**, 95 (2000).
7. A. Quereshi, S.K. Prabu and H.J. Purohit, *Microbes and Environment*, **16**, 49 (2001).