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Study of a new test paper for approximate estimation of phenolic substances

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Abstract
Phenolic substances are one of the major pollutants present in liquid wastes. Gas - liquid Chromatography and Ultra-Violet Spectroscopy, widely used for their detection and estimation, both require costly and sophisticated instrument and expertise. A simple sensitive and inexpensive Test paper for estimating phenolic substances is hence propounded. 2,6 chloroquine, 4-chloramine in benzene is used as a chromogenic reagent to yield a colored indophenol dye. The sensitivity of the estimation at ppm level.

Keywords: phenolic substances, test paper, method reliability

Introduction
Water is a valuable commodity available in a limited way. Phenolic substances are the most prevalent form of water pollutants present in the wastes of the chemical industry. Domestic, industrial and natural activities can lead to presence of phenolic substances in liquid wastes. Variety of hydroxyl benzenes and substituted hydroxyl benzenes constitute phenolic substances. The major discharge of phenolic substances is from paper. plastic pharmaceutical, textile and coking industries as well as petroleum refineries.
Discharge of phenolic substances affects normal utility of receiving water bodies since a carbolic odor is imparted to the water. Its regular consumption leads to severe pain, vomiting and capillary damage. Toxic effects are felt in the brain, lungs, kidneys, liver and pancreas even aquatic flora and fauna do not remain unaffected. The undesirable pronounced effects can be encountered even at a concentration as low as 0.001 mg/L. against the maximum permissible limit of 0.002mg/L.
Macherey-Nagal [2] has been marketing several indicators, test papers and test sticks for the detection of a number of inorganic pollutants in drinking water and industrial waters. However, no effort has so for been made to develop the same for the detection of organic pollutants. The methods commonly used so far for the estimation of phenolic substances have been gas liquid chromatography and ultra-violet spectroscopy, which are costly and sophisticated, Besides, they cannot be used for field detections, which may be often required Hence, an attempt has been made to develop a new simple, sensitive and inexpensive test paper for the approximate estimation of phenolic substances.
Feigl [3] has described five different procedures based on the formation of colored dyes (blue, red green, brown and yellow) for the detection phenolic substances, however, no application of the same has been made so far.

Experimental
In the present studies a chromatographic paper strip impregnated with sodium hydroxide, when spotted with a phenolic solution and dipped in 2,6- dichloroquinone-4-chloroimine (Gibb's reagent) in benzene has been found to turn blue.
The color formation is because of the reaction of phenolic substance with 2,6 dischloroquinone-4-choroimine producing yellow colored indophenol dye, which turns blue in alkaline medium. The intensity of the color as developed on the test paper can be estimated by a densitometer and then the concentration of phenolic substance computed.

Apparatus and material
Whatman No. 1 chromatographic paper strips (12×2.0cm) watch glass (8cm d), graduated micro pipette (00.01 ml) with vaccupet control, temperature controlled electric oven and densitometer (Systronics, India make) were used.
2,6-dichloroquinone-4-chloromine (Sigma, USA), phenol (BDH, India) 1-naphthol (BDH, India), Sodium hydroxide (Ranbaxy Ltd, India) and all other reagents and chemicals used were of analytical grade.

Solutions
Solution of 2,6 dichloroquinone-4-choloroimine in benzene ((0.1%) was used for impregnation paper strips.
Preparation of the test paper: - the paper strips (12×2.0cm) were cut into five equal parts and the centre of each part was marked in order to place the test solution there. The strips as obtained were impregnated the aqueous sodium hydroxide for 30 seconds. The excess solution was drained off by placing the strips over a filter paper sheet and allowed to dry at room temperature. The dry strips were collected in a hard paper box and used for testing phenolic substances.

Procedure
Detection by test paper
The test solution was spotted on the strip using a micro-pipette. The solvent was allowed to evaporate and color was

developed by dipping the test paper in a watch glass containing the reagent. The color so developed was recorded. To determine the lower limit of detection, the same volume (.01ml) of standard solutions of varying concentration of a particular test material was spotted on the test paper by maintain a distance of 2 cm between two spots. The solvent was evaporated and the color was developed and recorded as above.

Approximate estimation by the test paper

The same volume (0.01) of five standard solutions equivalent of 25,50,75,100 and 125 µg was spotted at five different and marked places. An equal volume (0.01) ml) of an unknown solution was spotted at the sixth place on the same test paper. The distance between two spots was 2 cm. The solvent was removed and the color was developed as above. Approximate estimation was made on the basis of comparison of the intensity and nature of the color of the spots.

Desitometric estimation by test paper

The same volume (0.01ml) of different standard solutions of varying concentration of a particular test material was spotted on the test paper and color was developed as above. the absorbance of the colored spots was read by the following procedure. the test paper was mounted on the glass plate and placed in a carriage of the Densitometer. The carriage was inserted in the slot till the geared rack engages the pinion side. The absorbance with yellow filter was recorded by advancing the carriage mm by mm. A calibration curve was made by plotting the concentration (µg) versus absorbance of the spot and it was used to determine the concentration of an unknown sample.

Results

The results of detection by the test paper are summarized in table 1. the lower limit of the detection of phenol (0.5µg) with the other phenolic substances is given in the same table. The results of approximate estimation of phenolic substances are summarized in table2. The results of densitometry estimation of phenol and their analytical parameters are given in table 3. The following expressions are used for calculating the analytical parameters.

$$\sigma = \frac{\sqrt{(x_1 - \mu)2 + (x_2 - \mu)2 + \cdots..}}{N - 1}$$

$$C.V. = \frac{\sigma \times 100}{\mu}$$

Where X_1, X_2 = measured values; µ= Average value

N = number of sets; σ = Standard deviation; C.V. = Coefficient of variation.

Table 1: Detection of Some Phenolic Substances by Test Paper

Test Material	Color Developed	Color Developed
Phenol	Blue	0.50
1-Naphthol	Blue with violet tinge	0.06
2-Naphthol	Violet	0.30
Catechol	Blue with violet tinge	1.20
Thymol	Blue	1.0
Resorcinol	Reddish violet	0.50

Table 2: Approximate Estimation of Some Phenolic Substances by Test Paper

Test Material	Concentration and color* Developed				
	25µg	50 µg	75 µg	100 µg	125 µg
Phenol	LB	LB	B	DB	DB
1-Naphthol	LBVT	BVT	DBVT	DBVT	DBVT
2-Naphthol	LV	LV	V	DV	DV
Catechol	LBVT	LBVT	BVT	BVT	DBVT
Thymol	LB	LB	B	B	DB
Resorcinol	LRV	LRV	RV	DRV	DRV

*Abbreviation used are: L= light; B= Blue; V=violet; D= dark; R=red; T=tinge

Table 3: Reliability of Colour of Phenol

Phenol	N	Absorbance	
µg		µ ±σ	C.V.
25	5	0.90 ± 0.037	4.1
20	5	0.88 ± 0.041	4.7
15	5	0.86 ± 0.038	4.5
10	5	0.85 ± 0.036	4.2
5	5	0.83 ± 0.031	3.8

N=No. of sets; µ= Average value; σ = Standard Deviation and C.V. Coefficient of variation.

Conclusion

On the basis of results, it is concluding that test paper established and proposed for the detection and estimation of phenolic substances is simple, sensitive and inexpensive. It can also be used as a field detection method. The sensitivity of the method can be increased on coupling with a suitable PR concentration method (extraction). The intensity of the color increases with the increasing concentration of the test material. Standard reference cards will have to be prepared or made available based on the above in order to avoid the need of a densitometer.

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