Forensic analysis of phenolphthalein-A Review
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Citation

Abstract
Although a number of different techniques using different chemicals such as fluorescent dyes, starch powder, phenolphthalein powders etc have been adopted but phenolphthalein powder in anticorruption cases has remained most popular in India. Phenolphthalein is a weak acid; its unionized molecules are colourless while on ionization give pink colour. The sodium carbonate washings containing traces of transferred phenolphthalein from accused is submitted to Forensic Science Laboratories (FSLs) for examination. There are so many conventional and instrumental techniques (Thin Layer Chromatography, UV-Visible Spectrophotometer, High Performance Liquid Chromatography, High Performance Thin Layer Chromatography etc.) which may be used for the confirmation of phenolphthalein.

INTRODUCTION
Phenolphthalein is being used in most of the anticorruption cases. Phenolphthalein powder, a smooth white powder is generally applied in small quantity on the currency notes and other objects are likely to come in contact with hands, pockets etc of the culprit resulting in its transfer (Locard’s principle of exchange).

The object (hand, bag, pocket etc) are washed with a colorless solution of sodium carbonate (or sometimes with lime water), which becomes immediately pink confirming the touching of currency notes/ transferred of phenolphthalein to the object. Pink to deep red colour is developed depending upon the quantity of phenolphthalein involved. These washings are being collected and sent to the forensic laboratories along with other relevant materials for analysis.\(^1\)\(^2\)

CHEMISTRY OF PHENOLPHTHALEIN
Phenolphthalein is a weak acid and is almost unionized. Its unionized molecules are colourless whilst on ionization give colourless H\(^+\) and pink coloured phenolphthalein ions.

In the presence of acid due to increase in the concentration of common ions (H\(^+\)), the dissociation of phenolphthalein is suppressed and thus the solution becomes colourless.

While the addition of strong bases (e.g. KOH, NaOH ) produces OH\(^-\) ions combine with the H\(^+\) ions from the phenolphthalein to form the ionized water. Thus more phenolphthalein ions are produced giving the pink colour solution.\(^3\)

Figure 1

Take appropriate amount of hand wash in a 100 ml beaker. Acidify this solution with dilute hydrochloric acid (till acidic to congo red), extract the solution with 3x30 ml diethyl ether. The extracted residue was crystallized with hot water as white crystalline mass (M.P. 213 °C). Concentrate the ether extract and use for further analysis.
MATERIALS AND METHODOLOGY

THIN LAYER CHROMATOGRAPHY:
The ether extract as above, was studied by Thin Layer Chromatography (TLC) using silica gel plates. Standard phenolphthalein was used for comparing. The plate was developed in a suitable solvent system and then sprayed with the suitable spraying reagent given below. TLC plate was exposed to ammonia vapour for visual development of undecomposed phenolphthalein as pink spot. It was reported that ammonia as spray reagent for the detection of phenolphthalein is neither sensitive enough to detect the traces nor the pink spot developed by it is stable and hence is unsuitable for routine analysis in Forensic Science Laboratories. Alternatively all the spots should be visualized by iodine fumes treatment of the developed plate and compared with the standards.

Mobile Phase:
- Benzene: Dioxane: Acetic acid (75: 15: 10)
- Chloroform: Acetone (4:1)
- Ethyl acetate: Methanol: Ammonia (80:10:5)
- Stationary phase: Silica Gel G

Visualizing reagent:
- UV light
- Exposed to ammonia vapour
- Spray with neutral ferric chloride solution.
- Iodine fumes
- With 1 % solution of potassium permanganate in 0.25 M sulfuric acid.

Diazocoupling reagent – prepared by mixing 25 ml of p-nitroaniline solution (0.3 % w/v) in hydrochloric acid (8%w/v) with 1.5 ml of a 5 % w/v solution of sodium nitrite in water followed by 10 % w/v of sodium hydroxide solution in water.

UV-SPECTROSCOPY
Take UV spectra of this compound in aldehyde free alcohol gives absorbance maxima at 225 and 285 nm. A Normal UV spectrum of phenolphthalein is known to show maxima at 277 nm in methanol. It was found that the first and second derivative UV- spectra of the ethanolic solution is more informative and is more specific.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC):
The ether extract were separately evaporated to dryness in glass capsules on water bath. The residue in each capsule was dissolved in about 1 ml of methanol and these separately prepared methanolic solutions of exhibit and control were subjected for HPLC studies.

Mobile phase of isocratic solvent system comprising of water: methanol (75:25) was used. A flow rate of 1 ml / min and UV absorption detection at 277 nm at ambient temperatures were maintained throughout the experiment. Integrator conditions were kept at Attn-1, Chart speed-1cm/min, Threshold = 2, peak width = 0.04.

Under these conditions we can detect phenolphthalein up to 1µg in anticorruption cases.

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC):
The concentrated / evaporated ether extracts from the different case exhibits, one control sample of phenolphthalein and one blank sample were separately spotted (5 ml each) as band (for better resolution) by using an auto sampler on dried and precoated silica gel 60 F – 254 HPTLC (20x20 cm) glass plate.

Solvent system of chloroform: acetone (9:1) was used for development of plate up to a distance of 10 cm. The developed and dried plate was studied on HPTLC densitometer for maximum absorbance at 220 nm and densitometric scanning between 200 nm to 300 nm wavelengths for spectrum of each extract.

After development of the HPTLC plate, spots were located under UV visualization chamber by absorbing in short wave UV region at 254 nm. One of the spot of each sample tallies with the control phenolphthalein.

The developed and dried HPTLC plate was also exposed to ammonia vapours. The pink spot of all the exhibits tallied with the control sample, which confirm the presence of phenolphthalein.

TROUBLESHOOTING IN TRAP CASES:
Phenolphthalein is known to give pink colour in alkaline at a concentration as low as 0.005 x 10^{-3} % (5µg per 100ml). The pink colour of this solution persists for some days to some
months depending upon the quantity of phenolphthalein and the strength of alkali solution. It gradually fades and some times becomes colourless at the time of trial in the court.

The fading of colour solution may be due to two reasons

- pH of the solution is out of limits
- Phenolphthalein has been broken into other colourless products.

In the first case colour can be regenerated by adjusting pH of the solution.

In the second case, this phenomenon can be explained on the scientific basis of that the colour of phenolphthalein fades due to its breaking down into 2 (4-hydroxybenzoyl) benzoic acid and phenol in alkali medium. The chemical break down of the alkaline phenolphthalein is caused by the oxygen present in the air. This can be prevented by the addition of hydroquinone which is strong antioxidant combines with the oxygen more rapidly than alkaline phenolphthalein does and thus retards the above reaction. It can be easily mixed with phenolphthalein powder before dusting the currency notes.

The presence of hydroquinone will provide another parameter to prove the transfer of money especially in those cases when the plea is taken by the culprit that he uses phenolphthalein tablets as laxative. The presence of hydroquinone can be easily established on the basis of chemical and instrumental methods.

Thus phenolphthalein used in trap cases is replaced by mixture of phenolphthalein and hydroquinone (100:2), the resulting pink colour does not fade appreciably after 4 years and can be seen by naked eye. Hydroquinone is a colourless powder and does not give pink colour in alkaline medium.  

**Figure 2**

CONCLUSION AND FUTURE PROSPECTUS

The coloured alkaline phenolphthalein solution unfortunately has a tendency to fade away with passage of time due to chemical changes. If the initial solution is highly coloured, with passage of time varying up to several months, its intensity may decrease but still it will be patently visible. On the other hand, if the alkali solution of phenolphthalein was only pink to light pink initially, because of very low quantity of transferred chemical it may become faint or even colourless after several weeks to months. Consequently when this physical evidence is finally produced in the court, often several months to year or more after the initial trap, the alkali solution may appear to be colourless or almost colourless. As the courts place reliance on the visual appearance of red colour of the alkali solution of phenolphthalein as a proof of transfer of phenolphthalein.

The absence or doubtful presence of phenolphthalein complicates matters. Defense often tries to take advantage of this situation. Although this problem has been solved by adding small quantity of hydroquinone (an antioxidant), but still more work has to be done in this area.

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References

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