DETERMINATION OF PHENOL LEVELS IN SOME SELECTED DISINFECTANTS BY SPECTROPHOTOMETRY TECHNIQUE

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ABSTRACT

The levels of phenol in some selected popular brands of disinfectants were analyzed in duplicates by molecular spectrophotometry technique. 5 g of each of the disinfectant sample was weighed into a 250 mL volumetric flask by difference and diluted to mark with distilled water. Single point calibration curve method was employed. Phenol standard was measured using a spectrophotometer at a wavelength of 510 nm to create a calibration curve. Subsequently, the concentrations of phenol in the unknown disinfectants samples were read off using the same spectrophotometer. Repeatability as the coefficient of variations is less than 3.5 % in all cases which connoted good precision and method performance. The analytical method is simple, sensitive, selective and highly reproducible and therefore reliable for routine analysis. The levels of phenol in the four disinfectants (Dettol = 5.75 %, Harpic = 4.00 %, Izal = 7 %, and Ivy's = 5.5 %) are found to be within the permissible concentration of 7% allowed by W.H.O. for disinfectants therefore unlikely to have any negative toll on the health of the end-users. Yet, there is the need for periodic monitoring of these products by relevant agencies to ensure the quality of these products and safety of the end-users.

Keywords: *quality, precision, single standard, and W.H.O*

INTRODUCTION

Disinfectants are antimicrobial agents containing phenol compounds that are applied to none living objects to destroy microorganisms that are living on the objects. Disinfection does not necessarily kill all microorganisms, especially resistant bacteria spores: it is less effective than sterilization, which is an extreme physical and/or chemical process that kills all types of life. Disinfectants are different from other antimicrobial agents such as antibiotics, which destroy microorganisms within the body, and antiseptics, which destroy microorganisms on living tissues. Disinfectants are also different from biocides; the latter are intended to destroy all form of life, not just microorganisms. Disinfectants worked by destroying the cell wall of microbes or interfering with the metabolism (Mid Sussex, 2009). Disinfectants find wide applications in domestic, industrial and hospital purposes.

Phenol, also known as carbolic acid, is an aromatic organic compound with the molecular formula C_6H_5OH. It is a white crystalline solid that is volatile. The molecule consists of phenyl group (C_6H_5) bonded to the hydroxyl group (OH). It is mildly acidic and requires careful handling due to its propensity to cause chemical burns (Aldrich, 2013). Many factories, such as coal conversion, petroleum refining, iron and steel, textiles, dyes, resins, plastics and agrochemicals, discharge wastewater containing phenolic or aniline compounds (Huang, 2005). It is a normal metabolic product excreted in quantities up to 40 mg/L in human urine (Wiley, 2003). Phenol and its vapours are corrosive to the eyes, the skin and the respiratory tract (Budavari, 1996). Repeated or prolonged skin contact with phenol may cause dermatitis, or even second and third-degree burns (Lin *et al.*, 2006). Inhalation of phenol vapor may cause lung edema (Budavari, 1996). According to Warner (1985), phenol may cause harmful effects on the central nervous system and heart, resulting in seizures, and coma. The kidneys may be affected as well. There is no evidence that phenol causes cancer in humans, according to U.S. department of health and human services. Phenol is also a reproductive toxin causing an increased risk of abortion and low birth weight indicating retarded development in the uterus. There is no scientific evidence or report that phenol causes cancer in humans (USDHHS, 2009). Beside its hydrophobic effects, another mechanism for the toxicity of phenol may be the formation of phenoxyl radicals (Hancha, 2000). The LD_{50} for oral toxicity is 300 - 500 mg/kg for dogs, rabbits, or mice; the minimum lethal human dose was cited as 140 mg/kg (Wiley, 2003). Due to its toxicity, they could have significant detrimental effects on water quality or animals as well as some plants even at very low level. For these reasons, some of them have been included in the lists of priority pollutants (Angerosa *et al.*, 1995). Chemical burns from skin exposures can be decontaminated by washing with polyethylene glycol, (Brown, 1975), isopropyl alcohol or perhaps even copious amounts of water. Removal of contaminated clothing is required as well as immediate hospital treatment for large splashes more especially if the phenol is mixed with chloroform.

Quality assessment of disinfectant in the society has faced serious neglect over the years as there is scanty literature found on studies carried out in this area. This work is poised to determine the concentration of phenol in some selected disinfectant so as to ascertain their quality in terms of effectiveness and safety of the end-users for domestic and hospital purposes.

MATERIALS AND METHODS

Sampling Technique

The convenient sampling technique was chosen with an emphasis on popular brands. Four different brands of disinfectants were purchased from a local market and small grocery retail outlets in Jalingo metropolis, Taraba State, Nigeria. All the brands of disinfectants were within their shelf lives at the time of the investigation.

Preparation of Solutions

All solutions and reagents were prepared following procedures adopted by AOAC, 1990.

Preparation of dilute nitric acid

Concentrated nitric acid was aerated until it becomes colourless; 1volume of it was diluted with 4 volume of distilled water.

Preparation of standard phenol solution

Phenol was first standardized using sodium thiosulphate after bromination with Winkler's reagent following procedures described by Ademoroti (1996). 6.1 g of the phenol (purity - 81.77 %) was diluted in 100 mL volumetric flask with distilled water to obtain 1 % solution and then diluted to make 0.025 % aqueous solution (Final Standard) daily.

Preparation of formaldehyde solution (Methanal)

2 mL of 37 % HCHO solution was diluted with 100 mL of water

Preparation of methyl orange indicator

0.1g of methyl orange (powder) was weighed into a 50 mL volumetric flask made to dissolve by shaking and made up to the mark with distilled water.

EXPERIMENTAL

Preparations of phenol standard and phenol blank for spectrophotometry analysis

5 mL of standard phenol solution was taken in two different boiling test tubes and then 5 mL of million's reagent was flowed downside of each tube, mixed, and tubes were placed in boiling water bath; boiled continually for 30 minutes, cooled immediately and thoroughly in a bath of ice water for 10 minutes and 5 mL of diluted HNO_3 was added to each of the tubes. It was well mixed and 3 mL of HCHO solution was added to each one pair of the tubes.

All tubes were diluted up to 25 mL mark with distilled water, stopper with cork, shaken and allowed to stand overnight. (Tubes containing HCHO faded to yellow; others showed red colour). 20 mL of each of the two phenol tubes were transferred into two different 100 mL volumetric flask using a pipette. 5 mL dilute HNO_3 was added to each of the volumetric flasks, diluted to volume and mixed (Red flask contained "phenol standard", the yellow flask "phenol blank").

Preparations of disinfectant samples for spectrophotometry analysis

Approximately 5 g of each sample of disinfectant was weighed by difference into a 250 mL volumetric flask and diluted to mark with distilled water (AOAC, 1990). 5 mL of aliquot prepared disinfectant solution was transferred into a 200 mL volumetric flask and promptly diluted with approximately 50 mL with distilled water. One drop of methyl orange was added to the solution and then dilute nitric acid until the solution was practically neutral, diluted to mark and shaken well.

5 mL of the prepared disinfectant solution was taken in boiling test tubes in duplicates to which 5 mL of million's reagent was flowed down the side of each of the tubes, mixed, and placed in a boiling water bath; boiled continually for 30 minutes, cooled immediately and thoroughly in a bath of ice water for 10 minutes and 5 mL of diluted HNO_3 was added to each of the tubes. After thorough mixing, 3 mL of HCHO solution was added to each one of the tubes. Each solution was carefully transferred to a 25 mL volumetric flask and diluted to mark using distilled water, corked and shaken well and allowed to stand overnight to develop colour. The resulting colour was red.

Principle

Phenol is a colourless substance but yet a chromophore owning to its π – electrons conjugated system of its benzene ring absorbs radiations in the UV/visible region of the electromagnetic spectrum at a shorter wavelength (about λ_{max} = 205). The attachment of auxochrome of NO_2^- via electrophilic substitution results in the formation of a coloured complex. This causes the absorption of the coloured complex to shift to a longer wavelength (510 nm), a phenomenon known as bathochromic shift (redshift) (Morrison and Boyd, 2006; Ambasta, 2013).

$$C_6H_5OH + NO_2^- \rightarrow \text{Coloured complex}$$

Spectrophotometry analysis of samples by single point calibration curve method

The levels of phenol in the disinfectants samples were determined following procedures adopted by (AOAC, 1990) with a slight modification of substituting the use of the Nessler cylinders (titration method) with a spectrophotometer (spectrophotometry method).

Single point calibration curve method was employed. Phenol standard was measured using a spectrophotometer, T60 2007 Model (made in the United Kingdom) at a wavelength of 510 nm to create the calibration curve. Phenol blank was initially used to zero the instrument in order to account for the absorption of other species other than phenol. Single point calibration curve method measures the concentration of phenol in the unknown disinfectant sample by finding the value of K in the calibration curve equation **C = K × Absorbance** from the single phenol standard of known concentration. The calibration curve is a straight line defined by the origin and the concentration of the phenol standard. Subsequently, the concentrations of phenol in the unknown disinfectants samples were read off using the same spectrophotometer. Distilled water was used for zeroing the instrument between readings of each of the disinfectants sample at the same wavelength.

Statistical Data Treatment

SPSS 16.0 Statistical and Microsoft Office Excel 2007 software was employed for the statistical data treatment. Analysis of variance (ANOVA) was carried out on replicate results obtained from the study. Repeatability of the coefficient of variation was calculated to checkmate indeterminate error.

RESULTS AND DISCUSSION

The result of the single point calibration curve method employed in the study is displayed in Table 1. The calibration curve equation is C = 0.26042 × Absorbance. The result of the analysis of the phenol levels in the four different disinfectants brands is shown in Table 2. In the set of replicate measurement for all the samples investigated no outlier was observed. Statistical analysis using analysis of variance show that there is a significant difference in the means of phenol contents in the different brands of disinfectants studied at $P < 0.05$ as shown in Table 3.

Table 1: Single standard calibration result for spectrophotometry analysis using phenol working standards

Working standard	Absorbance	Instrument reading (%)
Phenol blank	0.057	0.015
Phenol standard	0.096	0.025

Table 2: Result of the analysis of the phenol levels in the four different disinfectants brands using spectrophotometry method

Sample	Absorbance	IR %	Concentration = IR × DF (%)	Mean ± SD	CV %	Manufacturer's Label: Phenol conc.
Dettol 1	0.046	0.0120	6.000			
Dettol 2	0.044	0.0110	5.500	5.75±0.0007	0.012	
Harpic 1	0.033	0.0085	4.200			
Harpic 2	0.045	0.0080	4.000	4.1±0.141	3.45	
Izal 1	0.054	0.0140	7.000			
Izal 2	0.054	0.0140	7.000	7±0.000	0.00	7.00
Ivy's 1	0.045	0.0110	5.500			
Ivy's 2	0.045	0.0110	5.500	5.5±0.000	0.00	

IR = Instrument reading; DF = dilution factor = 500; SD = standard deviation

Table 3: Statistical analysis using analysis of variance (ANOVA) on result of replicates samples employed in the study

Replicates	Sum of Squares	Degree of freedom	Mean Square	F	Sig.
Between Groups	5.281	1	5.281	8.048	0.030
Within Groups	3.938	6	.656		
Total	9.219	7			

$P < 0.05$

Repeatability as the coefficient of variations are less than 3.5 % in cases, an indication of good precision and method performance (Westgard, 1998). In similar work by Phillips and Burns (2012) using RP-HPLC reported repeatability as the coefficient of variation in the range of 0.98 to 3.40 % for the free phenols, and 1.26 - 2.51 % for the salts, therefore, supported our findings in the present study. The concentration of phenol in the disinfectant samples was in the range 4.00 % - 7.00 %. Our results are comparable with those of Phillips and Burns (2012) where fourteen collaborating laboratories assayed o-phenylphenol (OPP), p-t-amyl phenol (PTAP), and o-benzyl-p-chlorophenol (OBPCP) in formulated products, both ready-to-use and concentrates, by RP-HPLC reported that actives in the samples ranged from 0.03 - 11 % OPP, 0.06 - 4% PTAP, and 0.07 - 10 % OBPCP either in free forms or as salts. The levels of phenol in all the disinfectants investigated are within the limit of 7 % allowed by W.H.O. (1987) for disinfectants and antiseptics. The results also show that the concentration of phenol stated in the product labels agree closely with the values obtained in this study. Unfortunately, some disinfectants manufacturers deliberately failed to include the composition of phenol or phenolic compounds in their labels while others had no composition details at all. Such products should be considered as unwholesome and the relevant agencies should keep track of them and confiscate these products with the view of possibly eliminating them from circulation.

CONCLUSION

The "Labelling of Poisons Act " makes it necessary to state the percentage of phenols present in disinfectants made from crude creosote, therefore, the employment of a simple method without cumbersome distillation process is obviously desirable. Our choice of spectrophotometry method in preference to the titration method in one of the stages of the experimental is advantageous as the colour of the complex formed darkened with time and unless the titration is carried out very rapidly the results obtained are likely marred in contrast with the use spectrophotometry method that takes significantly shorter time with more accurate and reliable results. Our analytical method was simple, sensitive, selective and highly reproducible and therefore reliable for routine analysis. The levels of phenol in the four disinfectants (Dettol = 5.75 %, Harpic = 4.00 %, Izal = 7 %, and Ivy's = 5.5 %) were found to be within the permissible concentration of 7 % allowed by W.H.O. for disinfectants therefore unlikely to have any negative toll on the health of the end-users. The phenol content as shown by the above test agreed closely with the amount stated on the manufacturer's label, thus showing that the test agrees well with the various methods adopted by manufacturers for the assay of their products.

Recommendations

Phenol has several detrimental health effects hence it is recommended that the quality of phenol in disinfectant should be properly monitored in order to minimize the effect that could be caused by the excess amount of phenol in the disinfectant. This information obtained from such monitoring exercise will help us to safeguard against adulterated disinfectants in circulation and take necessary measures to ensure that, such products do not get to the end users to avoid untold health hazards.

Competing interest

Authors have declared that no competing interests exist among them.

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