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Photochemical Reaction of Trichloroacetic Acid with Diphenylamine and Its Analytical Application

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A method for the determination of trichloroacetic acid (TCA) in a simple spectrophotometric way has been described. It is based on the photochemical reaction of TCA with diphenylamine (DPA) which forms a green colored product (λ_{max}: 652 nm). The relative standard deviation for all measurements is <7%. A linear calibration graph is obtained in the concentration range of 610 to 2875 ppm with a correlation coefficient 0.990. The method is simple, rapid and does not require any costly and hazardous chemical. The effect of solvent, reagent concentration, time of irradiation etc. have been discussed. Extraction of TCA from contaminated water or urine with upto 10 fold increase in concentration followed by photochemical reaction and absorption measurement showed good correlation with the theoretical value. The interference by chloroacetic acid (MCA), dichloroacetic acid (DCA), chloroform ($CHCl_3$), carbon tetrachloride (CCl_4), trichloroethylene (TCE), and perchloroethylene (PCE) have also been studied.

Trichloroethylene (TCE) and perchloroethylene (PCE) are two volatile, nonflamable solvents and have been used extensively in dry cleaning and metal cleaning industries. Metabolism of these solvents has been studied in both in vivo and in vitro systems and reported to produce trichloroacetic acid (TCA) and/or 2,2,2-trichloroethanol.[1] Thus, in order to obtain a relationship between the clinical state of a patient intoxicated with TCE or PCE and the concentration of TCA present in urine or blood serum, it is necessary to evaluate a selective, reliable and rapid method for determining this metabolite.

Moreover, during chlorination of potable water, various chlorinated organic solvent such as chloroform ($CHCl_3$), chloroacetic acid (MCA), dichloroacetic acid (DCA), and trichloroacetic acid (TCA) are reported to be formed in relatively large amounts in chlorinated water.[2—6] These by-products have caused much concern as hazards to health and need quick and easy method for quantification.

Several analytical methods have been reported which can be divided into procedures based on the colorimetric determination according to Fujiwara[7] and gas chromatographic methods coupled to specific detection techniques such as, electron capture detection (ECD),[8] mass spectrometric detection (MS),[9] microwave plasma emission detection[10] etc. These chromatographic separations were performed either on a strong polar phase of the underivatized trichloroacetic acid after extraction[11,12] or on a nonpolar phase either after extraction and derivatization[13—15] or vise versa.[16] Direct determination technique using high-performance liquid chromatography[17] and differential pulse polarography[18] are also known for TCA analysis. Also, various head-space gas chromatographic techniques[19,20] are known which involve decarboxylation or derivatization of TCA making them time consuming. All chromatographic methods are very sensitive but require expensive equipment, proper choice of column and specialized personnel. The spectrophotometric method due to Fujiwara lacks the selectivity and is extremely condition dependant.

During the last two decades, the photochemical reaction of aromatic amines like diphenylamine (DPA) with several chlorinated compounds like carbon tetrachloride (CCl_4),[21,22] polychlorinated biphenyls,[23,24] chlorinated pesticides[25] etc. has received considerable attention due to its simplicity and efficiency. But the reaction of DPA with chloroacetic acids remained completely unexplored.

Here has been reported, for the first time, the photochemical reaction of TCA with DPA in purely organic medium. The reaction produced a green colored product (λ_{max}: 652 nm) the absorbance of which has been used as a measure of TCA concentration in analytes. The method has the potential for quantifying TCA in water and urine samples. The most interesting feature is that the chloroorganic compounds which commonly occur with TCA such as, TCE, PCE, DCA, MCA, $CHCl_3$ do not respond to the reaction under the proposed reaction conditions. However, CCl_4 under the proposed reaction conditions gives rise to a red colored product (λ_{max}: 516 nm).

Experimental

Apparatus. Spectrophotometric measurements were performed on a Shimadzu UV-160 digital spectrophotometer. All photochemical reactions and the absorbance measurements were carried out in specially designed micro-sample quartz cells open at one end. The internal and external diameters are 2 and 7 mm respectively. The length is ca. 6 cm. These type of cells have the advantage in the sample limited situations[26] and should not hamper the determination. A portable germicidal lamp (15 W, Sankyo Denki, Japan) was used as the UV-source for the photochemical reaction. Gilson micropipette with disposable microtips was used for dilution and for taking aliquots into the cells.

Chemicals. All the chemicals and solvents used were of analytical grade and were used without further purification.

Water used was double distilled.

Standard Procedure of the Photochemical Reaction. 0.2-cm^3 portions of DPA (0.11 M in acetone, 1 M=1 mol dm^{-3}) were mixed with varying amounts TCA (0.017—0.08 cm^3, 0.1099 M in acetone) and then diluted with acetone upto a final volume of 0.5 cm^3. 0.14-cm^3 portions of the solutions were then taken into the quartz cells. The open ends of the cells were sealed with wax papers and placed under the UV-source for irradiation for 20 min. After that, the mixtures were vortexed for 1 min and allowed to stand for 15 min. Absorbance values were then noted at 652 nm. The measurements were done within 1 h after the UV-source had been removed.

Results and Discussion

Photochemical Reaction of TCA with DPA. Diphenylamine has been known to be an efficient sensitizer for many photochemical reactions[23,27] although the reaction of chloroacetic acids with DPA is hitherto unknown. In purely organic medium the photoirradiation of TCA with DPA led to a green-colored product. Figure 1 shows the absorbance spectra of the photoproduct (Curve A; reference: air) for the TCA concentration 1257 ppm. The graph shows an absorption maximum at 652 nm. The reagent blank after UV irradiation under the same conditions did not absorb at all in this range (Curve B; reference: air) and therefore for practical purpose no reagent blank was necessary. Irradiation of the reaction mixture with the light having short wavelength (ca. 254 nm) or visible light did not produce any color under these reaction conditions. Glass made capillary cells were not useful for this reaction.

Effect of Reagent Concentration. The relative concentration of the reagent with respect to TCA is important. For the concentration range of 610—2875 ppm for TCA a series of calibration curves were generated where the final concentration of DPA ranged from 0.022—0.088 M keeping all other factors constant. It was observed that 0.022—0.066 M DPA gave almost similar results. Further increase in concentration resulted tremendous decrease in the absorbance values of the photoproduct. So the concentration of DPA used in the reaction medium was 0.044 M.

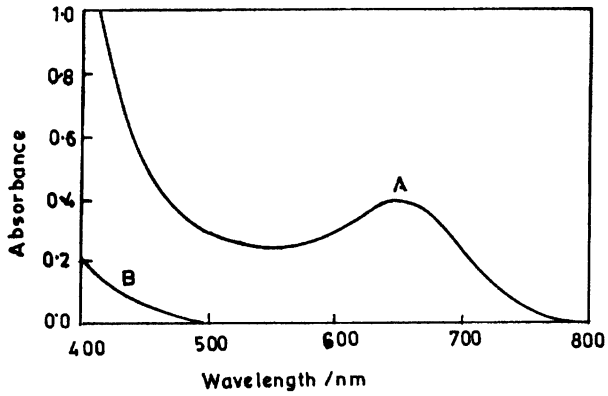

Fig. 1. Absorption spectra after 20 min UV irradiation of the mixture of DPA and TCA in acetone (Curve A) and DPA in acetone (Curve B). Reference used was air for both.

Effect of UV Irradiation Time. As the reaction is based on the exposure of UV light onto a mixture of DPA and TCA, the reaction is highly dependant on irradiation time. Hence a kinetic study was made for the system. It was noticed that shorter irradiation time not only leads to lower absorbance value but also tremendously effect the stability of the produced color. Irradiation time 20—30 min was found suitable for the development of maximum color and stability of the product. Further increase in irradiation time produced yellow color which hampered the measurement.

Stability of the Color. After the irradiation is over the intensity of the green color of the photoproduct decreased significantly for the first 15 min. Then it remained almost constant for at least 1 h. Hence all measurements were done after 15 min but before 1 h after the UV-source had been removed.

Choice of Solvent. Solvent plays an important role in the photochemical reaction. A thorough study was thus made with different solvents viz., acetone, ethyl acetate (EtOAc), cyclohexane (CH), methanol (MeOH), diethyl ether (Et_2O), dimethyl sulphoxide (DMSO), dimethyl formamide (DMF), ethanol (EtOH), nitrobenzene ($PhNO_2$), nitromethane (CH_3NO_2) and was observed that the reaction proceeds in MeOH, EtOAc, CH and Et_2O but in a less efficient way. In other solvents excepting acetone the reaction was inhibited totally. Acetone has been found to be the best-suited solvent and was used for the study. It has also been found that the photoproduct is quite stable in this medium in comparison to others. The possible reason for acetone to be a very good solvent is not well understood.[28] In the reaction mixture, the presence of EtOAc upto 20% was tolerated, whereas even 10% of MeOH, CH, Et_2O caused decrease in the absorbance intensity of the photoproduct. The reaction in the proposed conditions could not tolerate even 3% water.

Calibration Graph. A number of acetone solutions of DPA (final concentration: 0.044 M) and TCA (final concentration varied from 610—2875 ppm) were prepared and 0.14 cm^3 of each was introduced into the cylindrical quartz capillary cells. Irradiation of the mixture with UV light for 20 min and subsequent measurement of absorbance after 15 min at 652 nm were done. In this broad range of concentration, the plot of absorbance vs. concentration gave a linear correlation. The equation found for the calibration curve was $A = 5.839\times10^{-4}\times C(\text{ppm})-0.353$, $r=0.990$. Below a critical concentration, the absorbing species is probably present in the dissociated form. This was confirmed by adding strong HCl which increased the absorbance. Detailed study of the dependence of the absorbance on acid concentration is being published elsewhere. The relative

Table 1. Analysis of Water and Urine Samples

	Volume (cm^3) taken/used for		Concentration of TCA (ppm) present	Concentration of TCA found for the	% Error
	the spiked sample	the extraction	in the spiked sample	spiked solution[a] (ppm) ± S. D.	
Urine sample No.					
1	50	7	548	483 ±4.72	−12
2	10	2	818	744 ±10.01	−9
Water sample No.					
1	10	2	945	975 ±4.19	+3
2	20	3	630	710 ±3.22	+13

a) An average of five determination was taken for each sample.

standard deviation for all measurements was found to be <7%. The relatively high standard deviation could be due to the sample cell geometry.

Interference. To examine the selectivity of the method, a brief investigation was made with the possible interferring substances like DCA, MCA, $CHCl_3$, CCl_4, TCE, and PCE. None of them gave the photoproduct corresponding to the λ_{max}=652 nm under the stated reaction conditions. Although CCl_4 and $CHCl_3$ produce red colored product (λ_{max}: 516 nm),[22] $CHCl_3$ on 20 min exposure did not produce any significant color. On the other hand, CCl_4 although produces red-colored complex could be tolerated upto 4-fold excess. Under the proposed conditions, the determination of TCA was possible in presence of 5-fold excess of DCA, MCA, and $CHCl_3$, 3-fold excess of TCE and 2-fold excess of PCE.

Application of the Method for the Quantification of TCA in Water and Urine Sample. The method when coupled to a suitable extraction technique can successfully be applied for the analysis of TCA in water and urine samples. The extraction step also helps preconcentration of TCA form the water or urine samples and thus higher sensitivity could be achieved.

Extraction and Photochemical Reaction. A known volume of the spiked water or urine sample with known concentration of TCA was first made acidic with concd HCl (pH $\approx$ 0.5). The analyte was then extracted with EtOAc (known volume). Preconcentration of TCA in this extraction step can easily be achieved upto 10 fold or even more. The extract (0.1 cm^3) was then used directly for the photochemical reaction. It should be remembered that the reaction medium can tolerate only upto 20% of ethyl acetate. The photochemical reaction and subsequent measurement of the absorbance values was done following the standard procedure. The concentration was determined from the preestablished equation for the calibration curve. From these, the concentration of TCA in the spiked water or urine sample is found out. The results for two water samples and two urine samples are presented in Table 1. Under the conditions the results obtained showed satisfactory recoveries. Accuracy lies within ±13% which is comparable to that of the method due to Fujiwara reaction.[7]

Conclusion. A new photochemical reaction for TCA with DPA in purely organic medium has been reported. This yields a green colored product having λ_{max} at 652 nm. Spectrophotometric determination of TCA is possible at this wavelength. Although the proposed technique has lower sensitivity than Fujiwara method, it has unique specificity. Furthermore, the sensitivity can be increased following extraction technique. The important feature of this reaction is that PCE, TCE, MCA, DCA, $CHCl_3$, and CCl_4 do not produce this colored product. The method is simple, cost-effective, quick and reliable. The method could successfully be applied for urine and water samples.

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