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Digital camera analysis of dichlorvos by phloroglucinol and quantitate with standard colour chart in environmental water matrices – an approach

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Dichlorvos (DDVP) analysis using visible spectrophotometer, gas chromatography-mass spectrometry and liquid chromatography mass spectrometry has been studied extensively. The major drawback of all the method is requirement of standardisation, expensive and maintenance nature of instrument involvement. Since, many governments has banned the use of DDVP, standardisation is quite challenging with single point standard method. In this study, using digital camera based colour analysis for DDVP is carried out to develop one-time multistandard colour chart. The analysis is carried out by alkaline degradation of DDVP to dichloroacetylaldehyde to react with phloroglucinol and producing distinct colour gradients from pale purple to dark brown. The colour chart is able to quantitate the limit of detection and limit of quantification of 6 µg/mL and 20 µg/mL, respectively. The study is further experimented with real-time ground water with non-spiked and spiked DDVP samples for pre-concentration effect. The 1:1 linear relation is found with absorbance based spectrophotometer detection and digital camera image analysis to quantify around ppb level. This shows the colour analysis by human naked eye judgement is possible at certain level as semi-quantitate by strategical approach. In this paper, we have used the colour chart based digital camera detection of DDVP, which will be a viable alternate approach for analysing the environment samples.

Keywords: DDVP, Colour chart, Phloroglucinol, Semi-quantitative, Digital camera analysis

Dichlorvos (DDVP) is an organophosphorus (OP) insecticide and widely used to control the mosquitoes, cockroaches, crickets, ticks, leafhoppers, and other insects. Currently Indian government has banned the use from the year 2021, as they are hazardous to the environment and humans[1,2]. The left over DDVP residues was found in soil and water. The reported concentration in soil from India, Nigeria and Pakistan were 0.04 mg/kg, 233 mg/kg and 0.01 mg/kg, respectively [3]. Similarly, it was also reported in the water from China, India, and Thailand in the ranges from 10-50 ng/L, 90 to 800 ng/L and 1100 ng/L, respectively[4–6]. DDVP is readily soluble in water and able to migrate from soil and food matrices to human. The reference limit set by world health organization (WHO) for DDVP in drinking water was 20 µg/L. Similarly, the maximum residual limits (MRL) for food products such as rice & wheat grains – 7 mg/kg, vegetables – 0.15 mg/kg, fruits – 0.1 mg/kg, milk – 0.01 mg/kg and ground nut oil – 0.2 mg/kg. Hence, the recommended acceptable daily intake (ADI) of DDVP by humans was 4 µg/kg based on no-observed adverse effect level[7–9]. This shows the importance of DDVP monitoring analysis in various environment matrices for public safety.

The DDVP analysis was carried out by chemical extraction, separation of compound and analysis in the instruments. The detection limit (DL) of various instruments such as gas chromatography-mass spectrometry (DL – 0.016 µg/L)[10], capillary electrophoresis (DL –0.031 µg/L)[11], liquid chromatography tandem mass spectrometry (DL –1.5 µg/L)[12], high performance liquid chromatography (DL – 0.2 µg/L)[13], magnetic nanoparticle based immuno-fluorescent assay (DL – 70 µg/L)[14], luminol-H_2O_2 based chemiluminescence (DL – 0.8 µg/L) techniques[15], colorimetric based nanoparticles coated with polyacrylic acid on cerium oxide nanoparticles (DL – 8.6 µg/L)[16], ascorbic based Au nanoparticles (DL – 9.4 mg/mL)[17] and TMB (tetramethylbenzidine)-H_2O_2 based platinum nanoparticles (DL – 2.3 µg/L)[18]. After chromatography-based mass spectrometry, colorimetric based nanoparticles detection of DDVP is precise and helpful in human naked eye judgement. However, the challenges of nanoparticles-based detection were quite multifaceted due to synthesis,

stability, functionalization and interfering compounds has to be optimized, before environmental analysis[19].

The proven colorimetric based detection is spectrophotometric for DDVP is simple, fast and cost-effective method. But the detection limit is 10000 µg/L, which is quite higher. This method was reported by alkaline hydrolysis (pH:10-11) of DDVP and analysing dichloroacetaldehyde (DCA) via absorbance spectrophotometer using phloroglucinol reagent[20]. Similarly, resorcinol based alkaline lysis via absorbance has reported only in standards with 9400 µg/L[21].The major problem of analytical measurement in above instruments is the requirement of standards to quantitate the samples. Currently, DDVP is banned and hence, an alternative approach is required.

This paper proposes for DDVP analysis using colour to find out possibility inquantifiable using a method reported by Asthana *et al*.[20]. Phloroglucinol is an organic chromophores and the ability to produce orange colour formation with DCA and exhibiting maximum absorbance at 475 nm. The detection limit can be lowered by achieving increasing pre-concentration factor (PF) and results will able to reported by chromaticity[22]. The chromaticity method is colour space technique adopted by International Commission on Illumination (CIE) helps us to standardise with colour standards and make us standard reference colour chart. There are three distinct colour space methods available based on colour metric such as (i) Lab, (ii) LCH and (iii) LUV. Among them, Lab model is similar to human eye perception model to quantitate the colour. The function of Lab model depends on luminosity (L), a (green:red ratio) and b (blue:yellow ratio)[23].This helps us to understand the precise of the colour measurement based on tri-luminosity reported for point of care detection using digital camera and flatbed scanner[22,24]. In this paper, we standardise the colour chart using various DDVP standards and observe the analytical performance in real-time environment water samples. The proposed method will able to find out the colour tool can be either used as human eye for quick decision (semi-quantitative) or digital camera/visible spectrophotometer (quantitative) analysis for DDVP.

Materials and Methods

Instrumentation and reagents

Analytical measurements were performed in Shimadzu UV-visible spectrophotometer, Model: 1800 (Shimadzu Pvt. Ltd, Japan) and Canon Digital Still Camera, Model: Power shot SX400IS (Canon, India) with 16 megapixels, focal length-4mm, f-stop-3.4, exposure time-1/30 seconds and International Standard Organization (ISO) for camera sensors set for ISO200. Hot plate (Remi Pvt, Ltd, India) was performed as water bath temperature in 500 mL beaker. All the reagents were purchased from Loba Chem Pvt, Ltd, France, except 71% dichlorvos standard was obtained from Pi Industries, India. The standards were prepared in 99.9% of ethanol solvent as stock of 1 mg/mL. The working standards were 2.5, 5, 12.5, 25, 37.5 and 50 µg/mL. Phloroglucinol (1%) and sodium hydroxide (0.01 M, NaOH) was prepared in 18.2 mΩ cm resistivity of Type 1 MilliQ water. A known amount of DDVP concentration of 30, 40, 60 and 80 µg was spiked in 1 L groundwater (tap) matrices collected from our university campus.

Analytical procedure

This is an modified method used by Asthana *et al*.[20] in 2003. An aliquot of 0.5 mL DDVP standards was dissolved in 0.5 mL of 0.01 M NaOH in test tube. The tubes were kept in icy water condition under refrigeration at 4 °C for 20 min to undergo alkaline hydrolysis from DDVP to DCA. After incubation, 1 mL of phloroglucinol is added and kept in water bath at 70 °C for 15 min to form colour. Then the standards were kept immediately in icy water and further processed for visible spectrophotometer and digital camera image capture analysis. The real-time water (no spiked DDVP and spiked DDVP - 30, 40, 60 & 80 µg/L) samples of 1 L was extracted with diethyl ether of 200 mL portions in separating funnel and kept under hood for atmospheric evaporation overnight. The left over residues was washed with ethanol and transferred to test tubes. The remaining chemical steps followed same manner like DDVP standards, after dissolving 0.5 mL of 0.01 M NaOH in test tube.

Data processing and analysis

Data obtaining from visible spectrophotometer

The absorbance spectrum was collected from 400 to 700 nm of colour wavelength. Then the data was converted to transmittance and later into colour values. The colour values were processed using free software provided by Bruce Justin Lindbloom (http://www.brucelindbloom.com).

By this way, we have performed the absorbance and colour data from visible spectrophotometer.

Data obtaining from digital camera

The 10 mm path length glass cuvette was used as sample holder. The camera was placed in front of cuvette with a distance of 8.7 cm against the white background of Whatman Filter paper No 1 (not wall). The distance between the background (wall) and cuvette was 32.5 cm. This to avoid any shadowing effect created from general incandescent light source present in laboratory. The digital camera captured for 10 different images and performed image processing. The processing was performed by free image colour summarizer web service developed by Martin Krzywinski (Image Colour Summarizer Version 0.76, website: http://mkweb.bcgsc.ca/). The image process was carried out in k-means cluster statistical analysis from each image. The statistics parameters were set to 10 clusters, precision of pixel upto-200 px and colour data converted to text format to readout in excel for colour space calculation.

Colour spacing - Delta E (ΔE) analysis

In our study, we have processed visible spectrophotometer and digital camera image colour data to compute the Delta E (ΔE) analysis. This will able to calculate the colour space distance and compare the colours with CIE Lab values. The major reason of this mathematical model is similar process of human eye visual perception in colour analysis. The ΔE was calculated by Eqn (1).

$$\Delta E = \sqrt{((L_s - L_b)^2 + (a_s - a_b)^2 + (b_s - b_b)^2} \quad \ldots(1)$$

Where, L_s and L_b is luminosity from sample and reference, a_s & a_b is the colour ratio between red to green from sample and reference, b_s & b_b is the ratio between yellow to blue from sample and reference[25]. The CIE value is further converted into RGB model for data display representation.

Results and Discussion

DDVP analysis for colour Chart development using visible spectrophotometer and digital camera

The analysis of visible spectrophotometer is easy and possible to do colour analysis based on transmittance data. The relationship between transmittance and absorbance is shown in Eqn (2), which follows Beer-Lambert's Law [26].

$$A = \varepsilon bc = \text{In } (\%T) \quad \ldots(2)$$

Here, A is absorbance, directly proportional to ε (molar absorption coefficient), b (path length of light), c (concentration of analyte) and inversely proportional to transmittance. From transmittance data, colour data can be obtained in CIE format (CIE Lab) for analyte concentration. Since, colour is visible wavelength situated in 400 to 700 nm. The DDVP analysis was performed for colour chart standardisation in various concentration as shown in Table 1. The slope of colour based

Table 1 — Comparison of standardised colour chart for DDVP standard using digital camera and visible spectrophotometer

Std. conc. (µg/mL)	Colour reference chart for DDVP digital camera					Colour reference chart for DDVP using visible spectrophotometer					Colour differences between digital camera and visible spectrophotometer (ΔE)
	L (R)	a (G)	b (B)	Colour chart	ΔE value*	L (R)	a (G)	b (B)	Colour chart	ΔE value*	
Blank	47.99 (122)	13.00 (108)	-17.14 (143)		0	79.06 (196)	6.12 (177)	-3.08 (191)		0	28
2.5	48.99 (127)	9.56 (111)	-7.25 (129)		6.48	75.35 (187)	7.75 (164)	-3.48 (180)		3.12	23
5	47.36 (124)	9.93 (107)	-6.51 (123)		6.89	70.14 (171)	9.78 (146)	-8.03 (173)		8.09	20
12.5	46.75 (127)	8.85 (106)	2.05 (108)		13.10	75.22 (190)	8.39 (164)	0.96 (169)		4.89	25
25	44.33 (126)	7.90 (100)	15.15 (80)		23.76	74.35 (198)	10.19 (163)	14.35 (137)		13.91	27
37.5	43.22 (127)	7.30 (97)	26.45 (58)		31.28	71.45 (188)	11.23 (153)	10.68 (136)		12.57	28
50	40.66 (124)	8.70 (89)	33.52 (40)		34.61	71.47 (201)	13.88 (155)	29.85 (96)		22.18	29

*Each standard concentration (Std. conc.) analysis made to n=10 for reproducibility and repeatability of the data, ΔE value is colour spacing distance from blank (reference) to other standard (samples) concentrations

DDVP concentration for digital camera and spectrophotometer was 0.804 with r value of 0.943 and 0.431 with r value of 0.933, respectively. The detection limit, limit of detection (LOD) and limit of quantification (LOQ) for digital camera and visible spectrophotometer was 2 & 11 µg/mL, 6 & 33 µg/mL and 20 & 108 µg/mL, respectively, with 5-20% relative standard deviation in both analysis. Similarly, the slope of absorbance-based spectrophotometer DDVP concentration was 0.017 with r value of 0.999. The beer law is obeyed for the range of 2.5 to 50 µg/mL. The molar absorption coefficient and Sandells sensitivity were 4.19×10^3 L/mol cm and 0.0026 µg/cm^2, respectively. The chemical chromaticity plot shows the various DDVP concentrations in their distinct colour space quantified in visible spectrophotometer and transmittance data as shown in Fig. 1, which is validating for linearity in colour space with visible wavelength. The colour spacing model is developed by CIE, where the various colours of each wavelength from 400 to 700 nm have their unique tristimulus values. This helps us to distinguish the two-colour differences, where reference colour (note: blank as reference) is compared with sample colour (note: sample as standards).

In our study, we have used Lab model. Here, the various standards of DDVP have ΔE values as shown in Table 1 (ΔE- colour differences based on human eye perception), which is compared with blank. This value has made the linearity detection of our colour chart using ΔE Eqn (1) for unknown sample analysis. The same ΔE from Eqn (1) was used to compare the colour differences between the digital camera and visible spectrophotometer of Lab values from standards. This is to show the colour chart development is unique to each type of detection analysis with respect to illumination source (D value). The source used in digital camera detection is general incandescent light (room light (indoor daylight) – D50). In case of visible spectrophotometer, the source is halogen light (daylight – D65)[27]. Hence, developing the colour chart and detecting samples with either digital camera or visible spectrophotometer should be same. However, the advantage of colour chart standardisation is one time development, which is able to quantify many samples in a batch or semi-quantitate for different batch. The spectrophotometer is expensive and available only in laboratories compared to digital camera, which is ease in use and less expensive, but some laboratory standardisation of capturing images in any place is required.

The cost of chemical used in our analysis is cheap due to phloroglucinol versatility to detect the DDVP in our quantitative approach. Pomar *et al*. has reported the use of phloroglucinol in presence of chloride and ethanol, aromatic aldehyde can be coloured with pink to red[28]. Ivanov *et al*. has reported the capability using digital camera and office scanner might be possible to quantitate the chemical chromophores analysis for basic school/home level laboratories[22]. In our study, we demonstrated the use of digital camera for DDVP quantification and found to be slightly better than

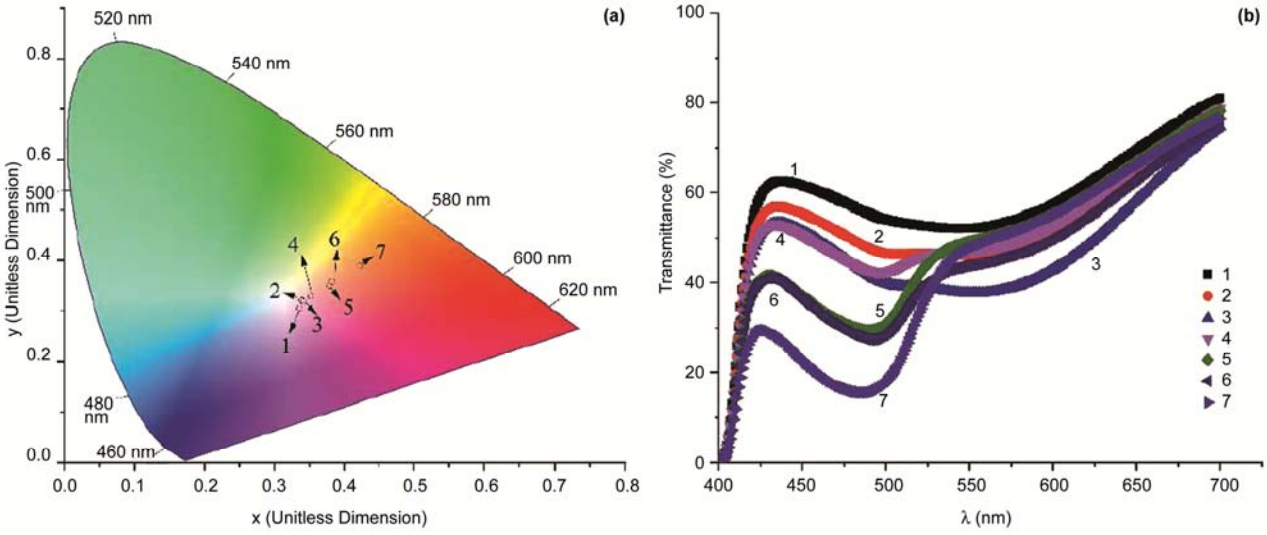

Fig. 1 — a) Chromaticity plot (CIE 1931) and b) Transmittance (%) data for DDVP Standard Concentrations from 1 - Blank, 2 - 2.5 µg/mL, 3 - 5 µg/mL, 4 - 12.5 µg/mL, 5 - 25 µg/mL, 6 - 37.5 µg/mL and 7 - 50 µg/mL

spectrophotometer in terms of colour analysis. The colour chart developed in our study is pale purple to dark brown, which act as chromophore and analysis will be look like pH paper. This is first time reporting the colour chart for DDVP quantification using phloroglucinol detection method. Based on our study, we analysed with real-time ground water samples along with few DDVP spiked samples for its capability.

Determination of DDVP in water samples using colour chart

The samples were analysed with both methods (visible absorbance and digital Camera based image analysis). The water samples were spiked in different concentrations and using diethyl ether, the recovery was observed above 70% as shown in Table 2. The concentration, we were able to detect was upto 24 µg/L in all methods, using pre-concentration factor correction (PF). The PF was made to 500 for detecting upto parts per billion levels. This approach was reported by Baskaran *et al*. to detect radionuclides[29]. The slope of 1:1 relation between both analysis in real time sample estimating DDVP was 0.9084 with r-value of 0.9541. The major advantage in using diethyl ether extraction was solvents can be evaporated to dryness. This made the turn-around time for pre-concentration was 1 day and analysis was able to achieve by 2 h for spectrophotometer (standards and water samples absorbance data as shown in Fig. 2b and 2c), 20 minutes for digital camera and quick decision for human naked eye judgement using colour standard reference chart as shown in Fig. 2.

The non-spiked water samples were not able to detect due to <LOD value. This shows the reliablity of the data with respect to spectrophotometer. The

Table 2 — Comparision of visible spectrometer and digital camera analysis data in drinking water

Water sample No.	DDVP spiked in 1 L water (µg/L)	DDVP Concentration with Pre-concentration Factor correction (µg/L)	
		Visible spec. analysis (CR%)	Digital camera analysis (CR%)
1	30	40 ± 0.22 (134%)	24 ± 1 (79%)
2	40	46 ± 0.47 (114%)	37 ± 2 (93%)
3	60	63 ± 0.79 (104%)	62 ± 3 (104%)
4	80	67 ± 0.91 (84%)	66 ± 2 (83%)
Blank	Non spiked	Not detected	Not detected

CR – chemical recovery of DDVP, all water samples performed in quadruplicates (n=4)

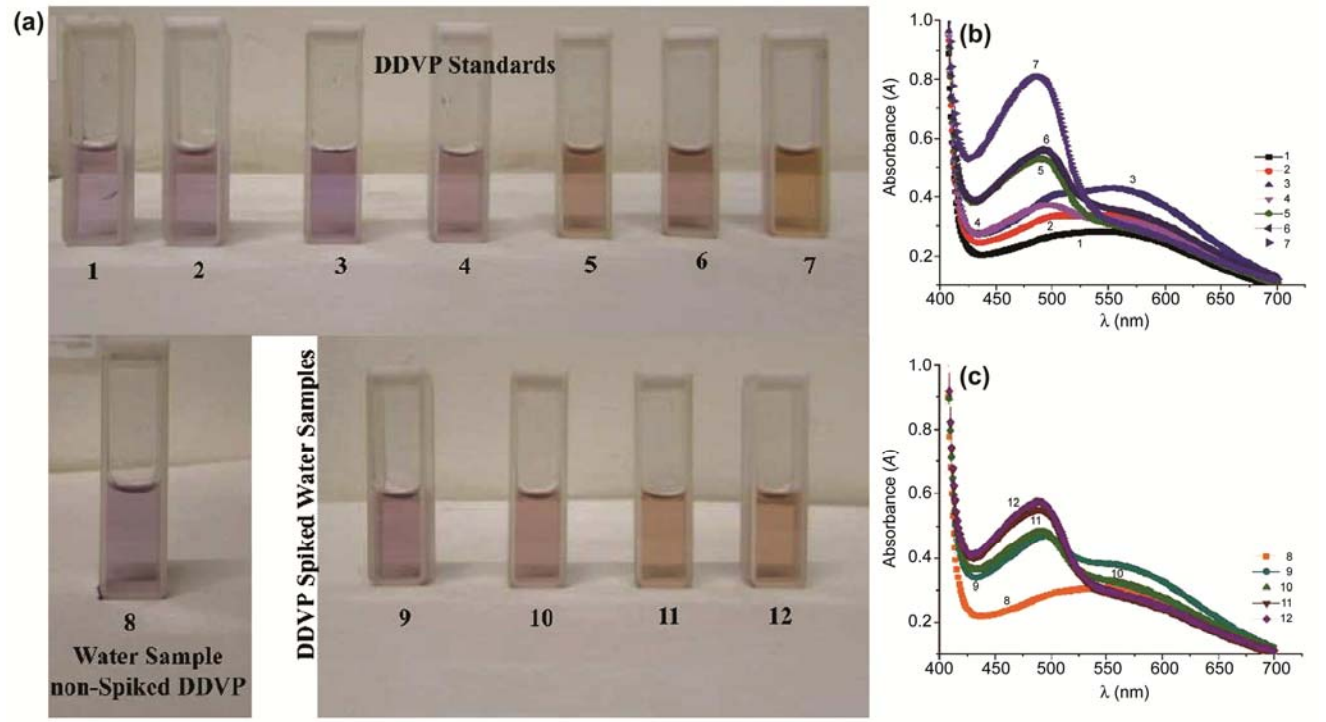

Fig. 2 — (a) Digital camera image of DDVP standards and samples in colour format for human eye interpretation, (b) Absorbance data of DDVP standards from 1 - Blank, 2 - 2.5 µg/mL, 3 - 5 µg/mL, 4 - 12.5 µg/mL, 5 - 25 µg/mL, 6 - 37.5 µg/mL and 7 - 50 µg/mL and (c) Absorbance data of Water samples from 8 – sample blank, 9 – sample 1, 10 – sample 2, 11 – sample 3 and 12 – sample 4

Table 3 — Comparison of analytical performance in the water samples with other reported colorimeteric methods for DDVP

Analytical method	Detection Type	Detection limit	Analysis time (min)	Colour range	References
Immuno- fluorescent based magnetic nanoparticles	Fluorescence spectrophotometer	4.75 to 8 µg/L*	>15	Fluorescence intensity	14
Luminol with H_2O_2 lysis	Chromatography based paper chip with luminescence analyser	3.6 µg/L	>12	Chemiluminescence intensity	15
Polyacrylic acid coated Cerium oxide nanoparticles	UV-vis Spectrophotomter	8.62 µg/L	30	Colourless to blue	16
Resorcinol with alkaline lysis		9.4 µg/mL	Few seconds	Colourless to yellow	21
Polydiacetyl vesicles with acetylchloinestrase and cationic surfactant	UV-vis Spectrophotometer & naked eye	6.7 to 50 µg/L	50	Blue to red	30
Acetone with alkaline lysis	Colorimeter& Infrared spectroscopy	Qualitative	120	Orange to red	31
Fluorescent based carbon dots-Cu(II)	X-ray photoelectron spectroscopy	840 µg/L	>3	Fluorescence intensity	32
TMB/H_2O_2 based platinum nanoparticles	UV-vis spectrophotometer	2.3 µg/L	3 to 20	Colourless to blue	18
Ascorbic functionalized Au nanoparticles	UV-vis spectrophotometer & naked eye	42.9 µM	Few minutes for naked eye	Red to purple	17
Phloroglucinol with alkaline lysis	UV-vis spectrophotometer	10 µg/mL	-	Orange	20
		40 µg/L*	120	Pale purple to pale brown	Our study
	Digital camera	24 µg/L*	20	Pale purple to dark brown	
	Human naked eye judgement	25 to 100 µg/L*	5 to 10		

*This value is based on preconcentration factor applied on the detection limit

limit can be even lowered by increasing the PF and able to detect the regulator levels imposed by WHO and food safety and standard authority of India (FSSAI) (20-150 µg/L)[8,9]. The present study is compared with other reported colorimetric methods for DDVP as shown in Table 3. The analytical performance of our study is nearer to the nanoparticle based colorimetric DDVP detection, but it works only after applying the PF corrections. From our study, we were able to do semi-quantitate DDVP using human naked eye judgement, i.e. from 25 to 100 µg/L based on colour standards to judge the various water samples. The naked eye judgement depends on individual's perception. Hence in our study, the digital camera detection was considered as quantitate. In this study, we have reduced the parameters such as cost, maintenance of instrument, synthesis, stability, functionalization and need of repeated standardisation. This is required in case of high end-instruments used in colorimetric based nanoparticle and chromatography based mass spectrometry detection of DDVP[10–15,19]. However, recently Indian government has banned the use of DDVP and we have shown the alternate approach for detection[2]. The use of colour chart in human naked judgement may provide an assistance for semi-quantitate analysis for environmental samples.

Conclusions

Based on our study, the use of digital camera for DDVP colour chart analysis was developed. The colour chart ranges from pale purple to dark brown as first time reporting. This will be ideal for real-time sample analysis as quantitative (digital camera image analysis) or semi-quantitative (quick decision by human eye) depending upon the purposes. The standard colour chart for DDVP analysis is developed for our laboratory like pH paper quantification. The LOD and LOQ for digital camera image analysis was found to be 6 and 20 µg/mL, respectively. However using PF as correction factor for real-time water samples can able to quantitate at 24 µg/L. The turnaround time was 20 min in analysis. But the sample extraction with diethyl ether was able to complete in 1 day. This may enable us to use the colour chart analysis as alternate options for other organic/inorganic analytes. In future studies, the colour chart has to be evaluated further in different laboratories for its efficient. However, recently Indian government has banned DDVP. So, this standardised

colour chart might be an alternative approach for semi-quantitative DDVP without DDVP standards for environmental samples.

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