mDSLR camera image analysis in universal pH indicator paper disk using various colour space models for quantitative approach

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Abstract

Universal pH indicator paper is frequently used to measure acids and alkaline in solutions. A digital single lens reflective camera captures the colour changes of the universal indicator, allowing for the determination of unknown pH values based on a colour chart. This study involved taking images of a 2 µL paper disk. Various colour models, including RGB, XYZ, Lab, Luv, LCH, CMY and HSL were analysed in the images. Among these, the Luv model was proven to be 96% to 98% of standard linearity in acidic and alkaline ranges respectively. The pH was effectively measureable from 4 to 12 with a resolution of 0.5.

The closeness of accuracy and precision shows 96.5% and 99.1% respectively nearer to a traditional pH meter measurement. In the present study, green and white analytical chemistry approaches were observed to be 99% and 92.6% effective for the 2 µL paper disk approach.

Keywords: Indicator paper, RGB, Luv, pH, digital camera.

Introduction

pH is an important analytical tool widely used for the measuring the concentration of hydrogen ions (H^+) and hydroxyl ions (OH^-) in an aqueous solution. The values of pH represent from less than 0 to greater than 14, which is logarithm reciprocal of the H^+ and OH^- concentrations in a solution. This is classified into three types such as acid, based and neutral. Value 7 indicates the neutral in H^+ and OH^- ions in the solution. Less than 7 indicates that the concentration of H^+ is increased called as acidic pH and greater than 7 indicates the concentration of OH^- is increased called as alkaline pH. This parameter helps for various sectors of biology, chemistry and physical applications ensuring quality product development in agriculture, health and industrial areas.

Currently, pH is majorly measured by the gold standard technique using glass electrochemical sensing via two-point standardization. The value 7 acting as blank helps in standardizing for both acid and alkaline pH regions i.e. any one value from both pH regions required for standardizing the respective regions. The electrometer has advantage due to the electrochemical potential for its accuracy and precise. However, the major drawback is that each-time separate standardization is required for acid and alkaline analysis. Thereby, the use of portability becomes problematic and low volume practically is impossible.

Many decades of developments have been made for portable electrometer by storing the standardization points in memory mode in the device for self-repeated standardization along with temperature adjustment detected by thermometer probe added in the device. To counter the electrometer device, indicator paper based pH was utilized. Indicators are potentially used in acid-base titration analysis to indicate the weak and strong solution through visual judgement. The chemicals such as azo, phthaleins, sulfonphthaleins and miscellaneous dyes acted as chromophores for indication. These dyes either single or multiple combination of dyes were used for pH indicator. As a result, the pH range is either specified or broad, ranging from 1 to 14. Each pH value is able to indicate a different colour as universal. That colour is specific to the pH value and it is universal chromophore phenomenon. The major advantage is ease of use, ability to understand the pH values on colours and a drop is enough for measurement. However, the major disadvantage is qualitative and lacks for the accuracy and precision. Therefore, the fluorescence based dyes were developed based on organic and metal organic dyes[16,18].

These probes were able to perform better than chromophores to provide the accuracy and precision for quantitative levels. But the requirement of fluors emission in dark condition is must for the estimation in optical analysis[15,16,19]. Many researchers have reported to use the chromophore based indicator using optical imaging method for quantitative approach. The use of digital camera is possible to capture the colours of indicator pH and ability to use the red (R), green (G) and blue (B) model to quantify. This model was developed by International Commission of Illumination (CIE) for colour space. Apart from RGB models, there are several models like Lab, Luv, LCH, HSL, CYM, XYZ etc.[3-5,6,7]

Most researchers have used RGB due to the camera image. However, this model is applicable for only electronic imaging or for display purpose in televisions and monitors level, not for visual perception. Similarly, each model has specific purpose such as XYZ is the mathematical model representation of colour plots in 2 dimensional view, X and Y act for primary colours and Z acts for luminosity. The cyan (C), yellow (Y) and magenta (M) are used for printer applications. The HSL and HSV is a cylindrical model, where the hue (H), saturation (S), lightness (L) and value (V) are used for computer graphics in image processing. In Lab, Luv and LCH where L is luminosity, a, b, u, v, C (chroma) and H (hue) are properties of primary colours. These models are used for visual representation of colours in human eye level. The pH indicator analysis was carried out specifically for human visual perception[10,19].

Apart from this reported universal indicator, pH was darker colour for quantification analysis using digital camera approach. This is easier to control in RGB models with distinct colour on each pH value from 1 to 14[5]. In this study, we would like to investigate the lighter colour based universal indicator pH paper analysis for quantifying the missing colour data of the pH values. Thereby, the accuracy and precision level were experimented in digital single lens reflective (mDSLR) camera using visual perception colour space models for optical quantification.

Material and Methods

Equipment and chemical reagents: The study was carried out with mirrorless digital single reflective lens camera (mDSLR, EOS M50 Mark II, Canon Inida Pvt. Ltd.). The acid reagent like 1 M of hydrochloric acid (HCl) and base reagent like 1 M of sodium hydroxide (NaOH) (Loba Chemie Pvt. Ltd., India) were prepared with 18.2 mΩ. cm resistivity of type 1 Milli-Q water (Merck-Millipore Pvt. Ltd., India). The certified reference material traceable to National Institute of Standards (NIST) of pH 4, 7 and 9 solutions were used in our study (Loba Chemie Pvt. Ltd., India). These reference materials were used in standardizing digital pH glass electrometer (pH Cal, Analab Pvt. Ltd, India) with resolution of 0.01 pH and accuracy to ±0.02 pH. The reference standards were also used to evaluate our method for validation purposes.

Fabrication of pH universal indicator paper disc and digital camera setup: The universal pH indicator paper disc was prepared from the Loba Chemie Pvt. Ltd., India from 1 to 14 colour chart standards provided by the company with 1 pH resolution. There are missing colour pH values of 2, 4, 11 and 13. The paper strip was hole punched to prepare the disc with a diameter of 0.6 cm. The disc was placed into the embossed A4 paper of 9-well chamber with dimensions of 5 cm X 5 cm and well diameter of 1 cm with 0.2 cm depth. The chamber was placed in the surface of the table and mDSLR was kept at a height of 25 cm as shown in supplementary figure 1. The disc was only able to hold 2 µL solutions for homogeneity mixture of chemical with indicators[4,6].

Image Processing: The captured image was processed in Image J software by selecting in oval size and cropped finally. The cropped image was data processed for RGB measure in that software. The data was further processed for converting into other colour space models such as XYZ, Lab, LCH, Luv, CYM and HSL using Bruce Justin Lindbloom software (http://www.brucelindbloom.com).

The collected data was subjected to colour differences (ΔE) computation using the equation (1):

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

where L represents the luminosity, a and b are colour data from red to blue region. Similarly, the equation is applied for other colour space models with respective three colour factors[2,4].

Supplementary Figure 1: DSLR image capture

Analytical report on green and white chemistry: The study was conducted with analytical greenness report (AGREE) for the method performance as reported by Penna-Pereira et al[11]. This will help us to understand the low volume performance. Furthermore, we have compared with white analytical chemistry to find out its analytical performance between the conventional method as reported by Nowak et al[9].

Results and Discussion

Standardization of mDSLR camera image for colour chart: The lighter colour based universal indicator pH in 2 µL based disk was carried out using mDSLR image analytical system. Table 1 shows the colour datas of the chart provided by the company and reactions occurred. The mean differences between them was 5.96, which is observed with our naked eye. This is due to the material of the paper. The colour chart (1) is prepared in the photopaper and pH paper (2) is filter paper. Thereby, in our study, we performed the unknown analysis with pH paper (2) as it was standardised with regular standards.

The ΔE was utilised as quantification to measure the unkown pH. Thereby, the pH 7 is neutral as it divides the acid (H^+) and alkaline (OH^-) and considered as blank. pH 1 was 15.4 and pH 14 was 26.26 from neutral. The spread of the ΔE data was plotted in chromaticity plot in figure 1. The acidic from 1 to 7 is distinct from 7 to 14. The colours were olive green to pink and olive green to blue. This shows that the indicator was made of one or more chemical dyes as reported by the researchers. The expected dyes were thymol blue, bromothymol blue, methyl red, thymol violet, thymolphthalein and dimethyl yellow[14,16,20].

The acidic region dyes were thymol blue for 1.2 to 2.8 (red), methyl red for 4.4 to 6.2 (red), dimethyl yellow for 2.9 to 4 (red), thymol violet for 1.2 to 2.8 (red) and bromothymol blue for 6 to 7.6 (yellow). This made the colours from neutral to acidic region to be observed as from yellow to pink with lighter. Similarly, the alkaline region dyes were thymol violet for 7.2 to 8.8 (yellow) and 9 to 13 (violet), thymol blue for 8 to 9.6 (yellow) and thymolphthalein for 9.3 to 10.5 (colourless). This made the colours from neutral to basic region to be observed as from yellow to blue.

Table 1
Standardised colour datas obtained using mDSLR camera image capture

pH values	Standard data obtained from colour chart (1)				Standard data obtained from pH paper (2)				Colour Difference between 1 and 2
	R	G	B	Colour Chart and DeltaE (ΔE)	R	G	B	Colour Chart and DeltaE (ΔE)	
1	232.32	198.90	226.61	24.19	239.60	200.18	222.08	15.40	2.17
3	226.74	194.18	198.59	19.65	237.28	203.17	180.73	7.23	5.76
5	225.88	208.57	160.63	10.72	225.13	201.07	157.03	3.31	0.81
6	224.38	211.02	155.39	9.34	239.01	222.28	169.51	2.91	4.30
7	204.97	202.80	117.04	0.00	230.24	224.41	162.32	0.00	10.34
8	218.28	224.46	178.99	11.30	209.01	205.99	148.00	6.26	5.02
9	200.91	215.79	175.63	11.06	187.17	200.75	168.20	13.69	4.63
10	157.92	191.62	169.61	20.65	189.60	203.23	178.53	13.64	11.16
12	164.18	185.31	181.65	21.57	156.56	173.04	166.70	25.46	3.33
14	137.52	149.21	183.85	34.24	168.18	157.92	190.72	26.26	12.12

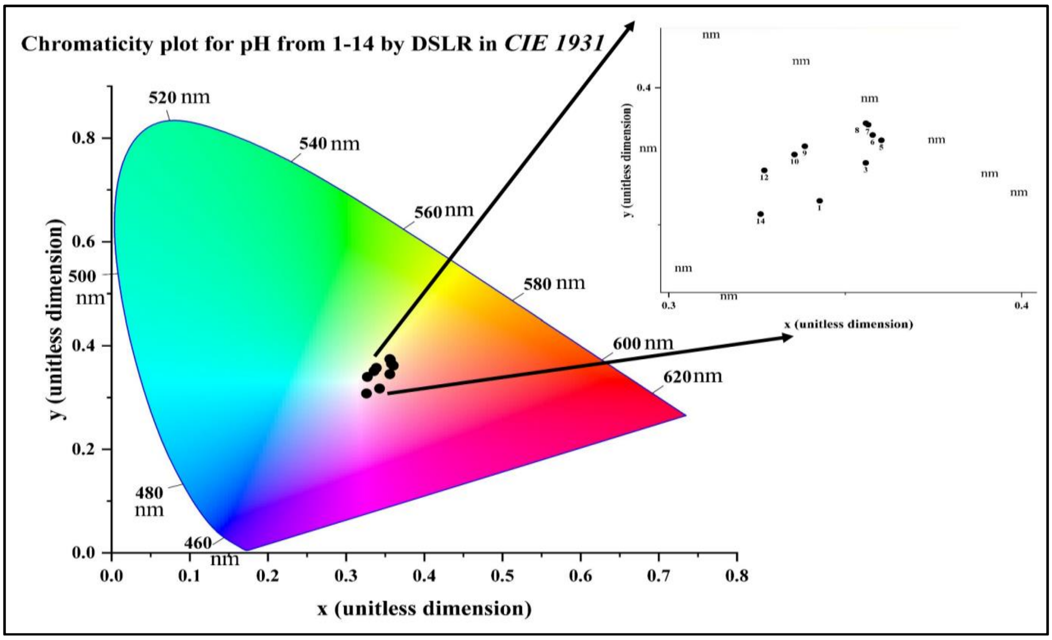


Figure 1: Chromaticity plot comparing (lab) our values

The pH can be quantified using the ΔE method as shown in table 1. However, the colour space has several models such as 1) XYZ a mathematical representation, 2) Lab, Luv and LCH as human eye model, 3) CMY as printing model and 4) HSL as colours of hue gradient based models. Among them, which is suitable for the quantification, has to be determined. Figure 2 shows the comparison of all the models using ΔE functions. The colour data values were shown in supplementary tables 1 to 3. This will be able to understand the uniformity between the points in which models are to be assessed for the quantification of pH.

The best model was found to be in this order Luv > Lab > LCH > HSL > RGB. The worst models were XYZ and CMY deemed to be unfit for the quantification. Even though the human eye model has 3 colour space, Luv was observed to be optimal due to the lighter colour. This lighter colour may be the mixture of thymolphthalein dye which is colourless. The luminosity in all three model is common. There is no increasing and decreasing trend due to the mixture of all dyes and behaved differently towards H^+ and OH^- ions.

Analytical Performance: The standard plot was plotted separately for acid and alkaline region as shown in figure 3a and 3b. The best linear fit was observed in Luv models in both regions. Table 2 shows the analytical parameter of each model to be considered for the quantification.

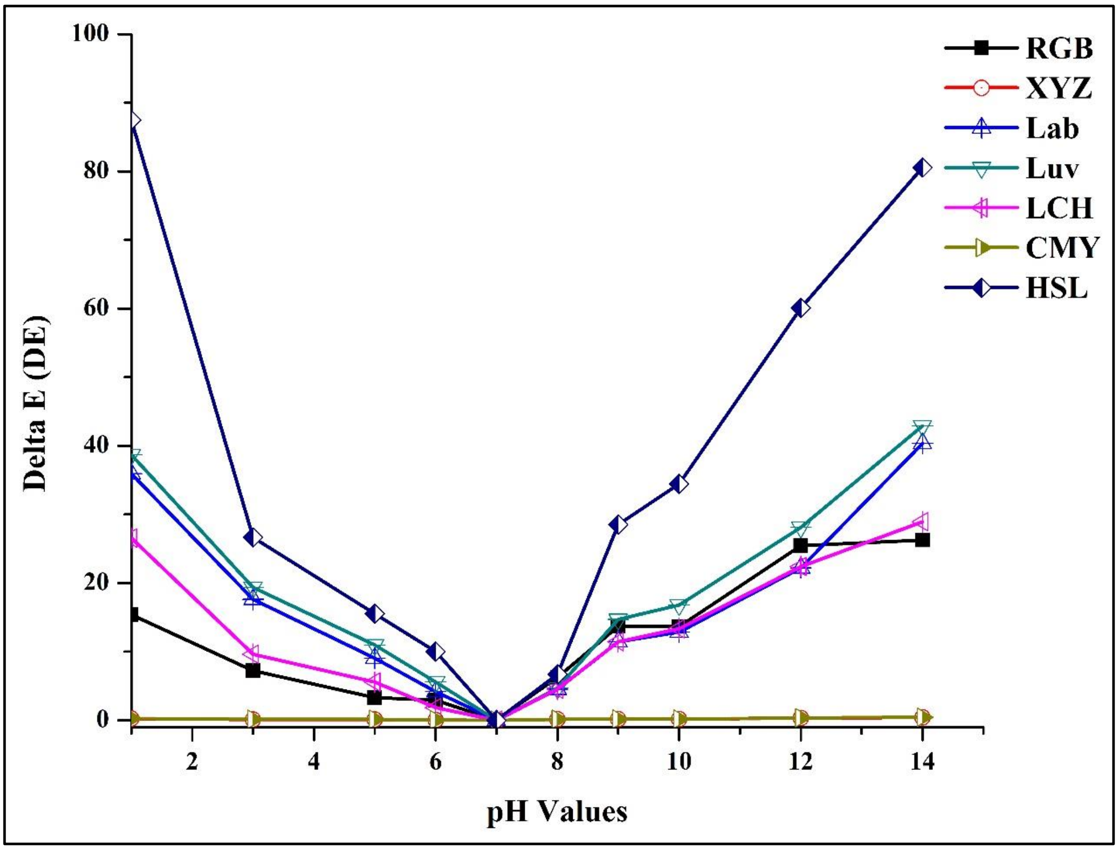


Figure 2: Comparison of all colour space models using ΔE method

Supplementary Table 1
Standardized colour datas of XYZ and Lab models

pH values	Standard data obtained from litmus paper of the brand (2)							
	X	Y	Z	Delta E	L	a	b	Delta E
1.00	0.88	0.81	0.87	0.25	84.55	17.47	-5.64	35.94
3.00	0.84	0.82	0.71	0.11	84.06	8.63	15.64	17.63
5.00	0.80	0.80	0.62	0.12	81.98	1.95	25.12	9.04
6.00	0.86	0.88	0.67	0.03	88.75	-2.11	28.00	4.17
7.00	0.84	0.88	0.64	0.00	88.46	-7.42	31.18	0.00
8.00	0.77	0.81	0.58	0.12	81.75	-7.95	29.30	4.52
9.00	0.74	0.78	0.66	0.16	79.09	-10.44	14.72	11.44
10.00	0.75	0.79	0.70	0.16	80.15	-9.12	10.74	12.84
12.00	0.64	0.67	0.65	0.33	69.33	-6.99	1.29	22.21
14.00	0.66	0.63	0.75	0.40	66.92	10.21	-15.53	40.35

The negative slopes were observed in the acid standard. This shows that the data was following the Henderson-Hasselbalch equation (2):

$$pH = pK_a + log_{10}\left(\frac{Base}{Acid}\right) \quad (2)$$

where the pK_a is the negative logarithm of acid dissociation constant. In the alkaline region, the negative intercept will be observed as it is opposite to acid region. This phenomenon was observed in the regular pH electro-meter.

However, in our study, this one-time standardisation helps the camera to guide using digital data for the unknown pH quantification. The present study measured the pH using the electrometer and analysed the solution with 2 µL universal indicator pH paper. Figure 4 shows the 1 to 1 analytical linear plot between the electrometer and universal indicator pH paper.

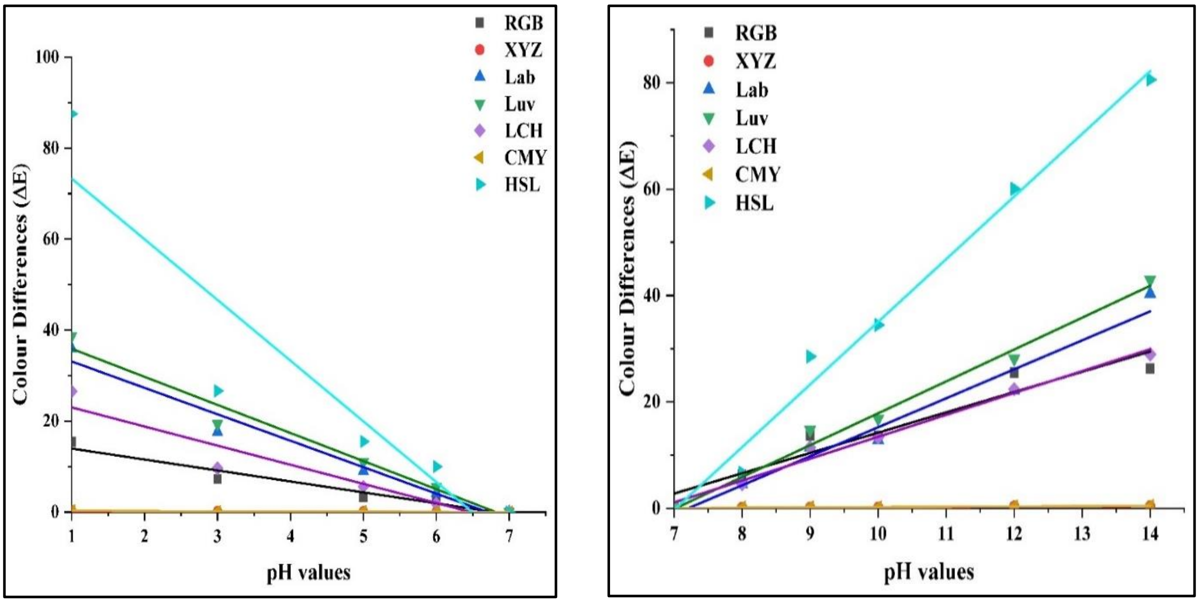
The analytical relation is able to understand the accuracy closeness and precision closeness based on the research reports[1,2]. The slope act as accuracy closeness and precision closeness act as adjusted r^2 value towards 1. The study was found accuracy closeness to be 96.5 % and 100% for Luv based mDSLR analysis and pH meter respectively. The precision closeness was found to be 99.1% and 100% for pH meter respectively.

This shows the universal based pH paper analysis using 2 µL was able to quantify. However, there are some disadvantage due to the mixture of five indicator dyes. The pH < 4 and > 12 are little far away from the analytical linearity line. Thereby, the pH from 4 to 12 is able to quantify 0.5 resolution using mDSLR camera analysis in our study as shown in table 3.

The mixture of indicator dye plays a role on chromophore towards the camera light. However, single universal indicator dye is better than the multiple as reported by many researchers[12]. The lighter colours will be specific challenge towards the camera light due to the reflectivity property.

Supplementary Table 2
Standardized colour datas of Luv and LCH models

pH values	Standard data obtained from litmus paper of the brand (2)							
	L	u	v	Delta E	L	C	H	Delta E
1.00	84.55	21.78	-11.75	38.69	84.55	18.36	342.11	26.56
3.00	84.06	22.58	20.84	19.36	84.06	17.86	61.10	9.62
5.00	81.98	17.66	34.37	10.96	81.98	25.20	85.55	5.55
6.00	88.75	13.27	39.50	5.55	88.75	28.08	94.30	1.86
7.00	88.46	6.82	44.48	0.00	88.46	32.05	103.38	0.00
8.00	81.75	4.86	41.45	4.68	81.75	30.36	105.18	4.55
9.00	79.09	-6.18	22.95	14.69	79.09	18.05	125.34	11.44
10.00	80.15	-6.50	17.24	16.78	80.15169334	14.09	130.34	13.36
12.00	69.33	-8.75	3.06	28.10	69.33	7.11	169.57	22.40
14.00	66.92	3.58	-25.22	42.92	66.92	18.59	303.33	28.93



3a: Acid region **3b: Alkaline region**
Figure 3: Linear standard plot for both pH regions

Supplementary Table 3
Standardized colour datas of CMY and HSL models

pH values	Standard data obtained from litmus paper of the brand (2)							
	C	M	Y	Delta E	H	S	L	Delta E
1.00	0.06	0.21	0.13	0.27	326.15	54.93	86.08	87.50
3.00	0.07	0.20	0.29	0.14	24.21	61.29	81.76	26.64
5.00	0.12	0.21	0.38	0.14	38.82	53.13	74.90	15.52
6.00	0.06	0.13	0.34	0.04	45.43	68.63	80.00	10.00
7.00	0.10	0.12	0.36	0.00	54.71	57.63	76.86	0.00
8.00	0.18	0.19	0.42	0.13	56.13	40.26	69.80	6.70
9.00	0.27	0.21	0.34	0.17	84.37	22.54	72.16	28.53
10.00	0.26	0.20	0.30	0.17	93.60	19.38	74.71	34.46
12.00	0.39	0.32	0.35	0.34	155.29	9.39	64.51	60.08
14.00	0.34	0.38	0.25	0.43	260.00	20.25	68.04	80.59

Table 2
Analytical capabilities of different colour space models

Parameter	Acid standard graph						
	RGB	XYZ	Lab	Luv	LCH	CMY	HSL
Slope + Intercept	-2.406 + 16.359	-0.0383 + 0.270	-5.808 + 38.914	-6.171 + 42.067	-4.201 + 27.207	-0.042 + 0.306	-13.340 + 86.631
Adj.R2	0.9255	0.8773	0.9561	0.9605	0.8740	0.8983	0.8120
Root Mean Square Error	1.628	0.0339	2.979	2.998	3.773	0.034	15.026
Reduced Chi square	2.650	0.0011	8.876	8.990	14.242	0.001	225.784
	Alkaline standard graph						
Slope + Intercept	3.816 – 23.948	0.053 – 0.341	5.451 – 39.285	5.994 – 42.087	4.117 – 27.726	0.057 – 0.370	11.781 - 82.757
Adj.R2	0.9015	0.9368	0.9567	0.9850	0.9837	0.9453	0.9854
Root Mean Square Error	3.253	0.036	3.008	1.922	1.376	0.035	3.723
Reduced Chi square	10.586	0.001	9.050	3.697	1.895	0.001	13.929

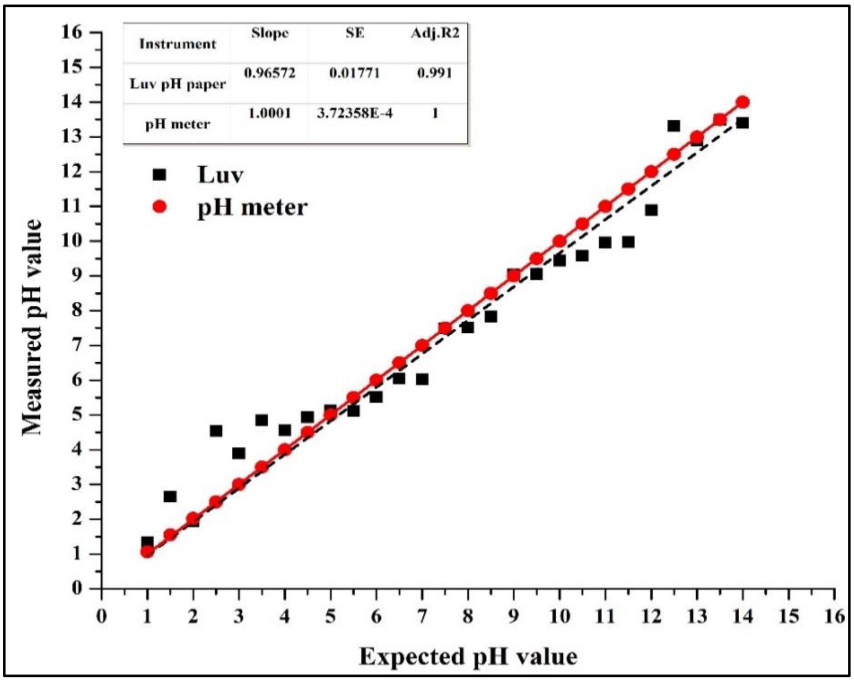


Figure 4: Analytical 1 to 1 linear plot between the electrometer and universal pH paper

Elsenety et al[5] have reported the 0.1 resolution using machine learning algorithm for the mobile camera > 5 megapixel. The indicator colours need to be darker in contrast to obtain the good quality signals using the RGB models [5]. The lighter colour on universal pH indicator has some limitations on chemical indicator dyes. The alternative solution was reported by Saeed et al[15] for use of natural based biodegradable dyes from betalains, anthocyanin and curcuminods, able to replace the conventional chemical based indicator dyes[15]. This will enhance greener and sustainable approach.

Analytical report on green and white chemistry: The AGREE report was based on the 12 principles of green and sustainable analytical chemistry. The closure to one is regarded as green and zero regarded as red. Figure 5 shows the differences between the pH meter and our study. The 2µL disk based universal indicator pH paper using mDSLR camera made the closure to 1. This is because of the volume of buffer solution, volume of chemicals, instruments and energy minimization. Similarly, the white analytical chemistry shown in figure 6 found that the pH meter was performing poor in the areas of energy, cost efficiency, time-efficiency, requirements like chemical and buffer solution and operational simplicity. In case of our study, the poor performing was found in the precision, accuracy and resolutions. Our study showed the 92.6% white and 65.3 % of white was found in pH meter. This shows the analytical sustainable is possible only by minimising the volume of solution, chemical, simpler instrument with energy saving[9,11].

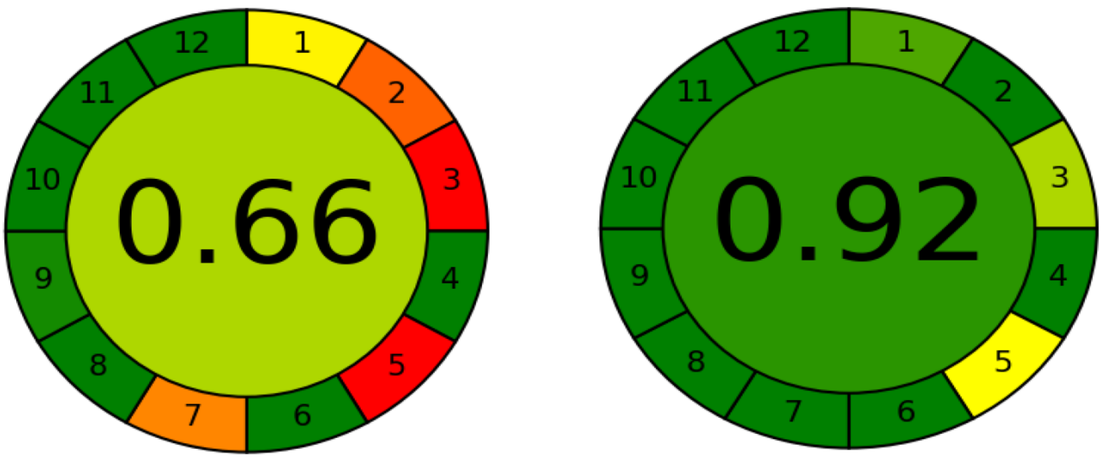
Conclusion

The present study found that the use of standardisation was important for using camera based analysis in universal indicator pH paper disk. The colour chart provided by the pH paper company is not good due to photo paper quality on colour reflective. The standardised colour data obtained from mDSLR on universal pH indicator paper was made of filter paper. The colour space models was good with Luv and poor in XYZ and CYM. The mDSLR was able to analyse 0.5 resolution of pH from 4 to 12. This is better than the other reported studies.

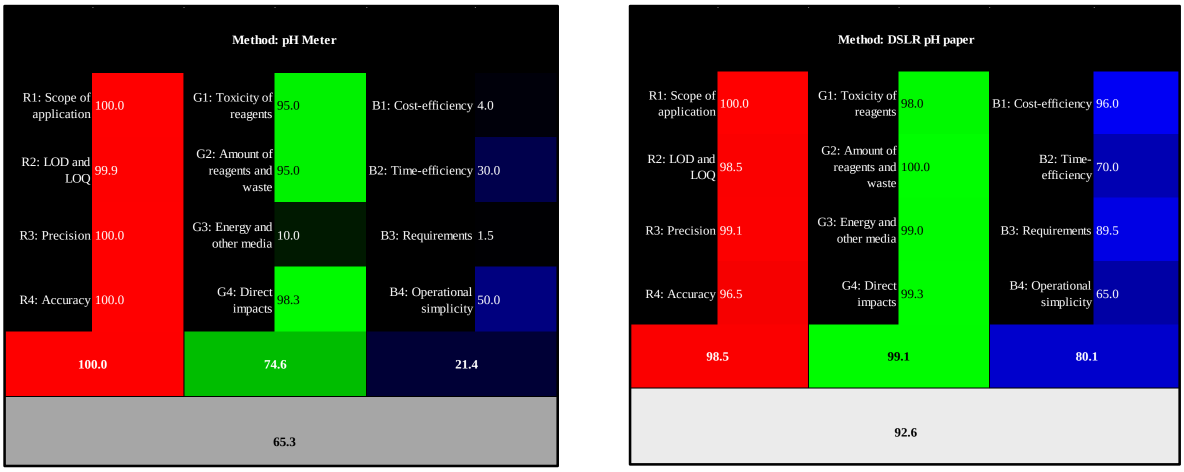
Table 3
Comparison of other reported studies

Type of Analysis	Colour Method	pH range	pH resolution	Turnaround time
Microfluidic digital camera 13 megapixel[9]	RGB	3 - 10	1	3 minutes
2 µL universal pH disc using mobile camera 8 megapixel[8]	RGB	1 - 6	0.5	1 hour
Smart app colour reader based on pH strip[6]	RGB	2 - 12	0.2	< a minute
Mobile camera greater than 5 megapixel[7]	RGB on machine learning	1 - 14	0.1	-
2 µL universal pH disc using mDSLR camera 24 megapixel*	Luv	4 – 12	0.5	5 minutes

*Our study



a) pH meter **b) 2 µL disk base pH paper by mDSLR camera**
Figure 5: Showing the AGREE between pH meter and our study



a) pH meter **b) 2 µL disk base pH paper by mDSLR camera**

Figure 6: Showing the white analytical chemistry between pH meter and our study

The major challenge is the mixture of indicator dyes with lighter colours. This will hamper the pH quantification. The universal indicator pH paper strip is qualitative for human based naked eye judgement. However, the mDSLR camera analysis might be able to quantitate the pH at certain levels. The analytical report on greenness was 99% and white chemistry was 92.6% for this study. The study suggests further developing on single indicator pH dye.

Acknowledgement

The authors would like to thank Dr. K.C. Patel Research and Development Centre and P.D. Patel Institute of Applied Sciences, Charotar University of Science and Technology (CHARUSAT) for providing all the facilities to conduct the research. Kainaz Daruwala expresses her gratitude for receiving CHARUSAT PhD Scholar's Fellowship (CPSF) by CHARUSAT and scheme of developing High quality research (SHODH) by Knowledge Consortium of Gujarat.

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(Received 28th December 2024, accepted 07th March 2025)