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Single drop scanometry determination of cyanide in water in the various color spaces

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Abstract

In this study, the application of new and simple single drop scanometric method was described for determination of cyanide as alternative for visible spectrophotometric method. Cyanide ion reacts with the methyl violet and causes a decrease in the color intensity of solution. In the single drop scanometry, color intensity of one droplet of solution was measured with color analyzing software in the various color models such as RGB, CMYK, HSV and XYZ. In the proposed method, characterization of single drop was done with potassium permanganate 0.001 M solution and pixel sampling box location, droplet location, glass plate location and image format were studied and optimized. This method has a linear range 5–12 mM with a limit of detection of 2.3 mM for CN^- ions. The developed method was successfully applied to the determination of cyanide in the mineral waters with acceptable results.

Introduction

Cyanide has excellent chemical properties and widely used in industrial fields such as making of plastics, recovery of gold and silver from ores, electroplating of metals (Hachiya *et al.* 1999; Baskin *et al.* 1997).

Cyanide is an inhibitor of the enzyme cytochrome c oxidase in the fourth complex of the electron transport chain (found in the membrane of the mitochondria of eukaryotic cells). It attaches to the iron within this protein. The binding of cyanide to this enzyme prevents transport of electrons from cytochrome c to oxygen. As a result, the electron transport chain is disrupted, meaning that the cell can no longer aerobically produce ATP for energy (Nelson *et al.* 2004). Tissues that depend highly on aerobic respiration, such as the central nervous system and the heart, are particularly affected. This is an example of histotoxic hypoxia (Biller *et al.* 2008). Cyanide poisoning is not common, but can more surprisingly occur from smoke inhalation from residential and industrial fires (Baskin *et al.* 1997; Moriya and Hashimoto 2001; Watanabe *et al.* 1997), where cyanide was released from the combustion of synthetic products that contain carbon and nitrogen, such as plastics and synthetic fibers. The extreme toxicity of cyanide and its great variety of uses in industrial applications make it crucially important to develop rapid, sensitive, selective and accurate method for cyanide determination. Many methods have been developed for the determination of cyanide, e.g. chromatography (Chattaraj and Das 1991; Nonomura 1987), atomic absorption spectroscopy (Chattaraj and Das 1991), spectrophotometry (Afkhami and Sarlak 2007; Gumus *et al.* 2000; Scoggings 1972; Cacace *et al.* 2007; Ohno 1989; Meeussen *et al.* 1989), fluorimetry (Gamoh and Imamichi 1991), selective electrode (Abbaspour *et al.* 2009; Gattrell *et al.* 2001). Cyanide ion reacts with the methyl violet according to the reaction of fig.1, and causes a decrease in the absorbance of the solution at 598 nm by spectrophotometric method (Afkhami and Sarlak 2007).

In the scanometric method, the sample solution was scanned with flatbed scanner, and then the image was transferred to image analyzing software. In the image analyzing software the color spots were analyzed to red, green and blue color values (Abbaspour *et al.* 2009).

In the present work, the application of methyl violet as a reagent for determination of cyanide based on single drop scanometry was described. After mixing sample solution containing cyanide with methyl violet, reaction was done and one droplet of final solution injected on the glass plate, before drying the droplet, glass plate was scanned with flatbed scanner. Image of solution single droplet was analyzed to color values in the various color spaces such as RGB, CMYK, HSL, HSV, XYZ, and correlation between any color values and concentration was studied.

To compare the spectrophotometric and scanometric method, it that should be mentioned, spectrophotometric techniques are based on the measurement of transmitted light by an analyte. In the visible spectrophotometric methods the sample must not be turbid and the species should often have a sharp λ_{max} to obtain the precise determination in the visible region. The linearity of the Beer–Lambert law is limited by chemical and instrumental factors. One of the instrumental deviations occurs when the incident radiation is non-monochromatic, which can be minimized by using λ_{max}.

But in the sacnometric method, which used in this research, the transparency of the solution in is not important, because the light reflection is measured, and the light radiation does not pass across the solution. Also, the sharp λ_{max} of the species are not important, because in this method the intensity of the color of the solution was analyzed by software to three main color (red, green and blue) values (Abbaspour *et al.* 2009).

Material and methods

Apparatus

A 4400F Canon scanner with a cold cathode fluorescent lamp (CCFL) and CCD (charge coupled device) as a light source and detection system respectively was used for scanning the glass sheet containing of sample solution droplets. The CCFL is a three wavelength source (for red, green and blue regions). The resolution of the scanner was regulated at 200 dpi. In order to inject sample solution on the flatbed scanner, a plate of glass with thickness of 1 mm was used.

The written software in VB 6 media converts the recorded pictures of color of cells to RGB (red, green and blue) data. A Brand micropipette was used for injecting samples into the cells. The pH values of solutions were measured by a Metrohm pH meter.

Reagents and chemicals

All of the chemicals used were of analytical reagent grade. Deionized water was used to prepare the buffer, reagents and stock solutions. To characterization of the single drop, potassium permanganate with concentration of 0.001 M was used. A 0.1 M stock of standard solution of cyanide was prepared by dissolving adequate amount of sodium cyanide (Merck) in deionized water. Phosphate buffer with concentration of 0.1 M was used for study of effect of pH. These solutions were kept in a dark, cold place and were freshly prepared every day.

Color analyzing principle

RGB color model (or color space) is an additive color model in which red, green, and blue light are added together in various ways to reproduce a broad array of colors. In computing, the color values are often stored as integer numbers in the range 0 to 255, the range that a single 8-bit byte can offer (by encoding 256 distinct values). In the RGB system, any color is represented in the form of (R, G, B), in which the (0, 0, 0) and (255, 255, 255) refer to black and white respectively (Abbaspour, 2011) [21].

Any color value (V) can be described by the following formula:

$V = R + 256G + 256^2B$

Where, R, G and B are red, green and blue values of the main color.

The CMYK color model is a subtractive color model, used in color printing, and is also used to describe the printing process itself. CMYK refers to the four inks used in some color printing: cyan, magenta, yellow, and key (black). To convert RGB system to CMYK system the following algorithm was used:

1. R' = 1 - (R / 255)
2. G' = 1 - (G / 255)
3. B' = 1 - (B / 255)
4. Black = min (R', G', B')
5. Cyan = (R' - Black) / (1 - Black)
6. Magenta = (G' - Black) / (1 - Black)
7. Yellow = (B - Black) / (1 - Black)

HSL (hue-saturation-lightness) and HSV (hue-saturation-value) are the two most common cylindrical-coordinate representations of points in an RGB color model. Developed in the 1970s for computer graphics applications, HSL and HSV are used today in color pickers, in image editing software, and less commonly in image analysis and computer vision. To convert RGB model to HSV and HSL system and calculate the H, L, I, chroma, S_{HSV}, S_{HSI} and V_{HSV}, following algorithm was used[22]:

1. Convert the R, G and B values to the range 0 - 1, by dividing each color value to 255.

Find min and max values of R, G and B.

2. I = (R + G + B)/3
3. L = (max + min)/2
4. V = Max (R, G, B)
5. Chroma = max - min
6. If the max and min colors are the same, S is defined to be 0, and H is undefined but in programs usually written as 0
7. Otherwise

SHSL = Chroma / (1 – |2L - 1|)

SHSV = Chroma/V

SHSI = 1-Min/I

8. For H

If R = max ⇒ H = [0 + (G - B)/(max - min)]/6

If G = max ⇒ H = [2.0 + (B - R)/(max - min)]/6

If B = max ⇒ H = [4.0 + (R - G)/(max - min)]/6

Also to calculate color values in the XYZ color system, the transformation matrix (M) was used as followed [22]:

$$[X\ Y\ Z] = [R\ G\ B][M]$$

$$M = \begin{bmatrix} 0.4124564 & 0.2126729 & 0.0193339 \\ 0.3575761 & 0.7151522 & 0.1191920 \\ 0.1804375 & 0.0721750 & 0.9503041 \end{bmatrix}$$

$$\Rightarrow \quad X = 0.4124564\ R + 0.3575761\ G + 0.0193339\ B$$
$$Y = 0.2126729\ R + 0.7151522\ G + 0.1191920\ B$$
$$Z = 0.0193339\ R + 0.0721750\ G + 0.9503041\ B$$

Results and discussions

Study and characterization of single droplet

To study and characterization of single droplet on the glass plate at the scanner surface, solution of 0.001 M potassium permanganate was selected. Some experimental parameters such as glass plate thickness, scanner resolution, image format, glass plate location, droplet location, and sampling zone and ... were studied and optimized.

Study the effect of glass plate thickness

In order to study the effect of glass plate thickness on the color values, two glass plates with thickness of 1 and 2 mm were prepared. Five droplets of solution of potassium permanganate 0.001 M were injected on the two plates and after scanning, images were transferred to color analyzing software. Results of this experiment were shown in the fig.2.

As fig. 2 shows, change of the glass plate thickness has no significant effect on the most color values, but the same glass plate was used in the next experiments.

Table 1. LOD of proposed method.

Color parameter	Detection Limit (mM)
R	2.6
G	3.7
B	2.5
C	5.2
M	2.3
%H	4.9
% S_{HSV}	3.8
Y	3.1
Z	3.2

Table 2. RSD% of proposed method.

Color parameter	RSD% (n = 10)
R	5.2
G	6.0
B	4.3
C	3.8
M	8.2
% H	5.8
% S_{HSV}	7.3
Y	3.7
Z	2.9

Study of the sampling box location in the single drop

In the color analyzing software, a box (with size of 40000 pixels) was used to analyze the specific location of image. To study of the effect of sampling box location on the standard deviation of pixels, one droplet of potassium permanganate (0.001 M) was injected on the glass plate. Then scanning was done, and in vertical and horizontal direction sampling was

done. Standard deviation of pixels was obtained, and results of red and blue values (as representative of other color values) are shown in the fig. 3 and 4.

As fig.3 and 4 show, in the edges of solution droplets, standard deviation between pixels in the sampling box increased.

Table 3. Interference study in the color parameters.

	Relative errors in the color parameter								
Interfering species	R	G	B	C	M	%H	$\%S_{HSV}$	Y	Z
IO_3^-	4.2	2.3	5.6	3.4	2.3	3.2	4.2	3.5	3.2
HCO_3^-	6.3	2.9	4.5	2.8	7.2	5.6	3.4	4.2	3.4
CO_3^{2-}	4.2	4.3	4.5	4.3	2.9	2.8	7.2	5.6	5.2
$S_2O_3^{2-}$	5.3	8.4	2.6	4.3	4.3	3.7	3.2	6.5	6.3
Cl^-	4.2	4.8	7.3	5.6	3.9	6.7	5.3	4.3	4.5
Br^-	2.3	7.6	8.5	4.6	3.9	4.8	4.7	5.4	6.2
SCN^-	4.2	4.8	6.5	2.7	3.8	4.8	5.6	6.4	5.4
Thiourea	3.3	6.3	3.5	5.7	8.6	4.9	5.8	6.6	4.9
S^{2-}	4.3	9.5	3.8	2.3	3.7	4.3	5.4	8.4	7.2
$S_2O_3^{2-}$	5.2	8.4	6.8	2.5	4.3	6.3	4.5	4.3	4.3
Mg^{2+}	3.7	3.6	5.4	4.3	5.4	6.7	2.5	3.2	5.3
Fe^{3+}	7.2	4.5	7.3	5.6	6.4	4.5	3.4	5.4	3.2
$Cr_2O_7^{2-}$	13	12.2	8.8	9.5	13.2	12.5	11.2	10	12

Table 4. Determination of cyanide in mineral waters.

	Relative errors in the color parameter								
Types of mineral water	R	G	B	C	M	%H	$\%S_{HSV}$	Y	Z
Sepidan	5.6	5.2	3.2	-2.3	3.7	-2.3	3.7	3.5	2.8
Sheshpeer	7.2	3.2	2.9	5.2	5.6	4.3	-4.2	-4.2	2.9
Kuhestan	3.4	6.2	3.4	4.5	6.2	3.6	-4.2	4.5	5.3
Mineral	6.2	4.3	2.9	5.2	2.7	3.2	3.8	4.5	3.4
Ivan ab	4.5	3.6	3.8	3.8	4.1	-4.2	3.5	3.9	4.5

Therefore in the central zone of each droplet sampling boxes have approximately same color values and low acceptable standard deviation.

Effect of resolution of scanner on the color values

In order to study the effect of scanner resolution on the color values, potassium permanganate 0.001 M was prepared and five droplets of this solution were injected on the glass plate. Fig. 5 shows color values for all color parameters at various scanner resolutions.

As shown in fig. 5, the color values in the all color models are independent from scanner resolution. Standard deviations of all color parameters are shown in the Fig. 6, as the result of this figure, the resolutions of 200 and 400 dpi have lower standard deviation.

Table 5. Comparison between the proposed method and other method for the determination of cyanide.

Method	Principle	Instrumentation	Figure of the merits
Optical sensor[11]	Based on the reaction between cyanide and immobilized methyl violet on a triacetylcellulose membrane	Spectrophotometer, Decrease in absorbance at λ = 598 was measured.	Linear range = 3.8-95 mM LOD = 2.4 mM
Single drop scanometry (this work)	Based on the reaction between cyanide and methyl violet in the solution	Scanner, color values intensity were measured.	Linear range = 5-12 mM LOD = 2.3 mM

In order to increase speed and decrease the volume of images, the resolution of 200 dpi was selected for next experiments.

Study of the effect of image format

To study the effect of image format on the color values, solution of potassium permanganate 0.001 M was prepared and five droplet of this solution were injected on the glass plate. After scanning, the image was transferred to color analyzing software. Fig.7 shows results of this study.

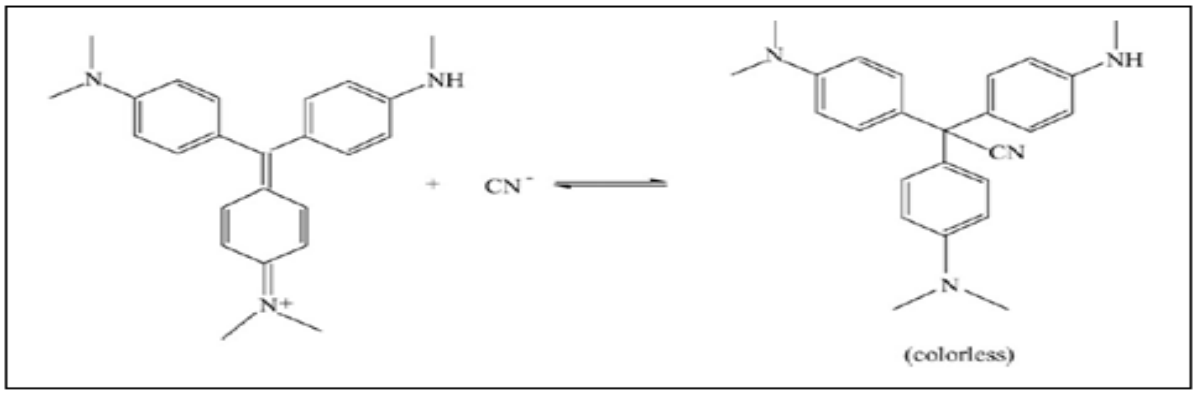

Fig. 1. Reaction between methyl violet and cyanide.

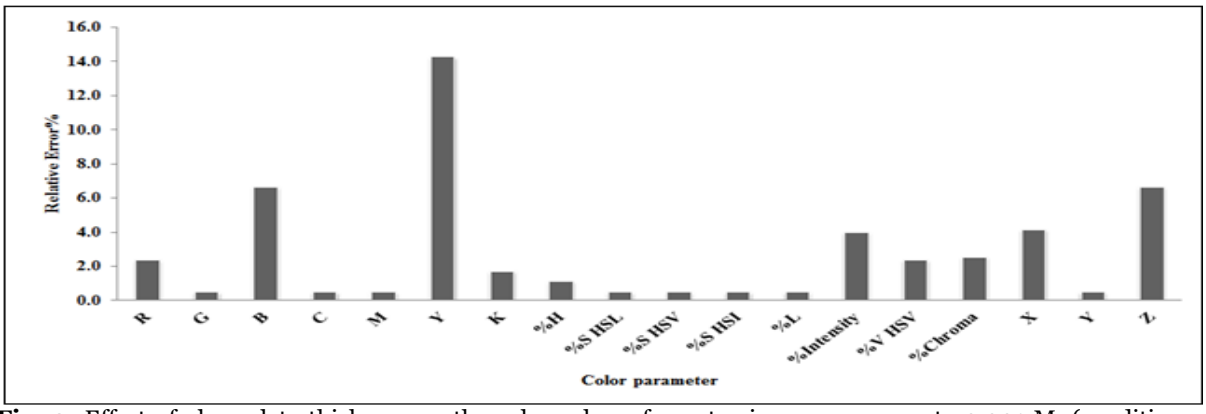

Fig. 2. Effect of glass plate thickness on the color values, for potassium permanganate 0.001 M, (conditions: format of images is jpg and resolution of scanner is 400 dpi).

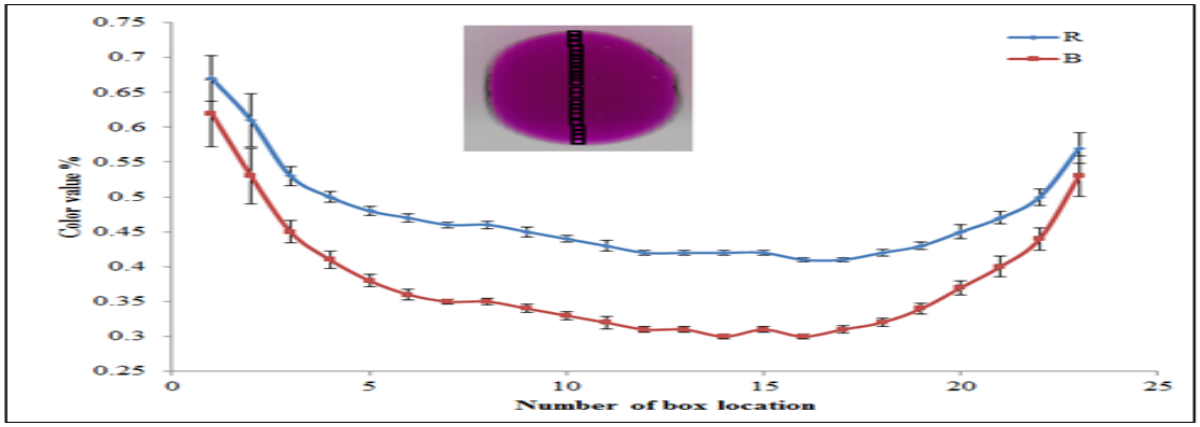

Fig. 3. Color values for red and blue in the various sampling box (vertical direction) for potassium permanganate 0.001 M, error bars indicate standard deviation in each box (conditions: format of images is jpg and resolution of scanner is 400 dpi).

As fig.7 shows, change of image format has no effect on the color values.Therefore to decrease the volume of images, the jpg format was selected for next experiments.

Study the effect of droplet location on the glass plate

The effect of location of droplets on the color values was studied as followed. A solution of potassium permanganate with concentration of 0.001 M was prepared and some droplets of this solution was injected on the glass plate at random locations. Fig.8 shows results of relative standard deviation of color parameters at various locations on the glass plate.

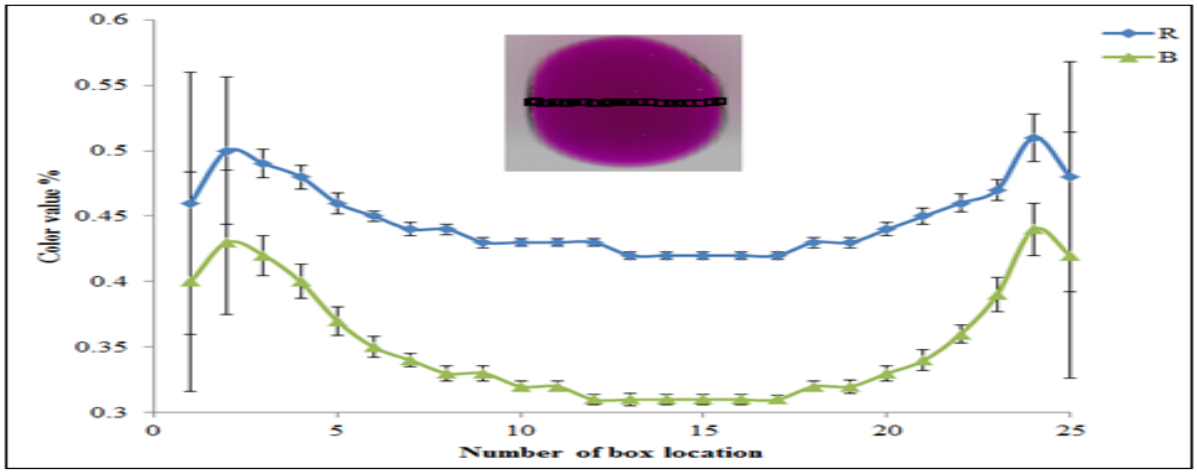

Fig. 4. Color values for red and blue in the various sampling box (horizontal direction) for potassium permanganate 0.001 M, error bars indicate standard deviation in each box (conditions: format of images is jpg and resolution of scanner is 400 dpi).

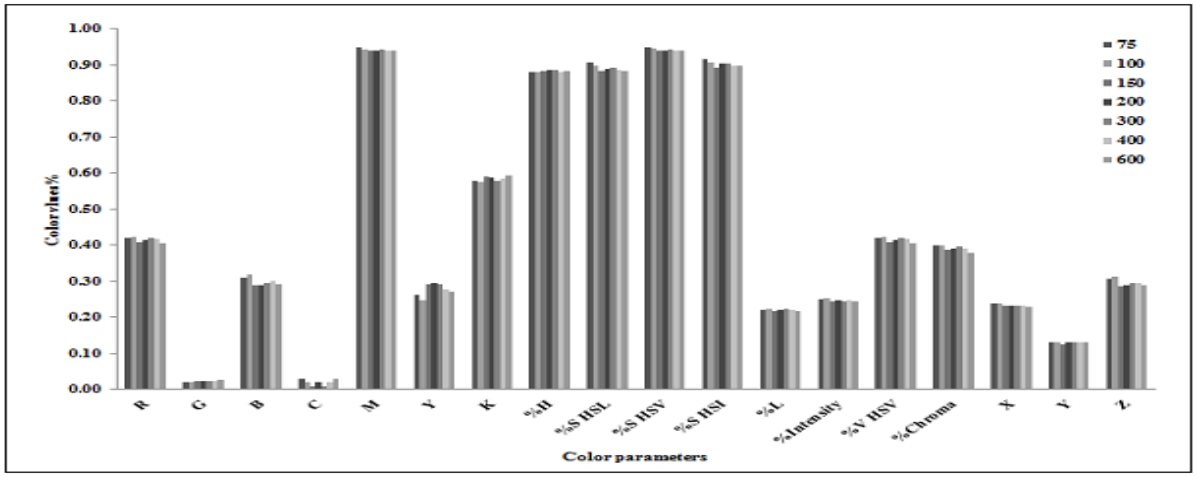

Fig. 5. Color values for all color parameters at the various scanner resolutions for potassium permanganate 0.001 M, (conditions: sampling zone is central, format of images is jpg).

As can be seen in fig.8, in most color parameters, % RSD is acceptable less than 5%, however in the next all experiments, fix and same location was applied for sample injection on the glass plate.

Study the effect of glass plate location on the scanner surface

To study the effect of glass plate location on the scanner surface, glass plate containing a droplet of 0.001 M potassium permanganate was scanned at the randomize various location on the scanner surface. Fig.9 shows relative standard deviation between

various glass plate locations. As this figure shows, for all color parameters (except green), RSD % between glass plate location was acceptable less than 10%, however at the next experiments fix and same location was selected for glass plate on the scanner surface.

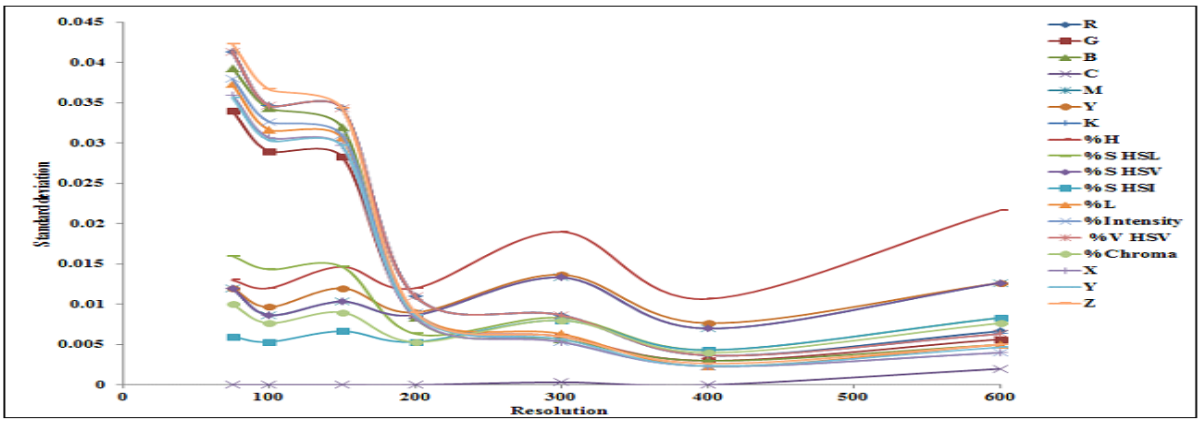

Fig. 6. Standard deviation of color values for all color parameters at the various scanner resolutions for potassium permanganate 0.001 M, (conditions: sampling zone is central, format of images is jpg).

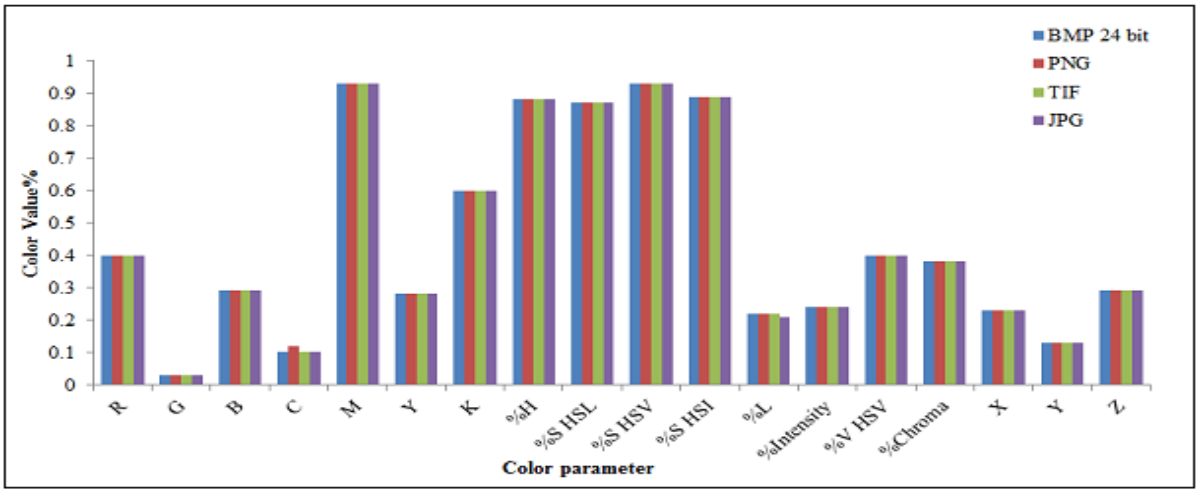

Fig. 7. Color values for all color parameters at the various image format for potassium permanganate 0.001 M, (conditions: sampling zone is central, resolution is 200 dpi).

Study the effect of pH on the methyl violet color parameters

In order to study the effect of pH on the color parameters of methyl violet, various solutions of this reagent with concentration of 0.01 M were prepared at different pH (1.5 – 13). pH of these solution were adjusted by phosphate buffer 0.1 M. Then five droplets of each solution at every pH were injected on the glass plat, after scanning the glass plate, images were analyzed by color analyzing software. Fig.10 shows the result of Red, Magenta, $\%S_{HSL}$ and $\%S_{HSI}$ as representative of all color parameters. As results of fig.10 show, color values of methyl violet is independent of pH between 2.5 – 11.

Study the effect of pH on the cyanide-methyl violet product color parameters

Cyanide ion reacts with the methyl violet and causes a decrease in the color intensity of the methyl violet solution. In order to study of the effect of pH on the reaction between methyl violet and cyanide, solution containing 0.01 M cyanide and 0.01 M 0.1 M methyl violet were prepared at different pH.

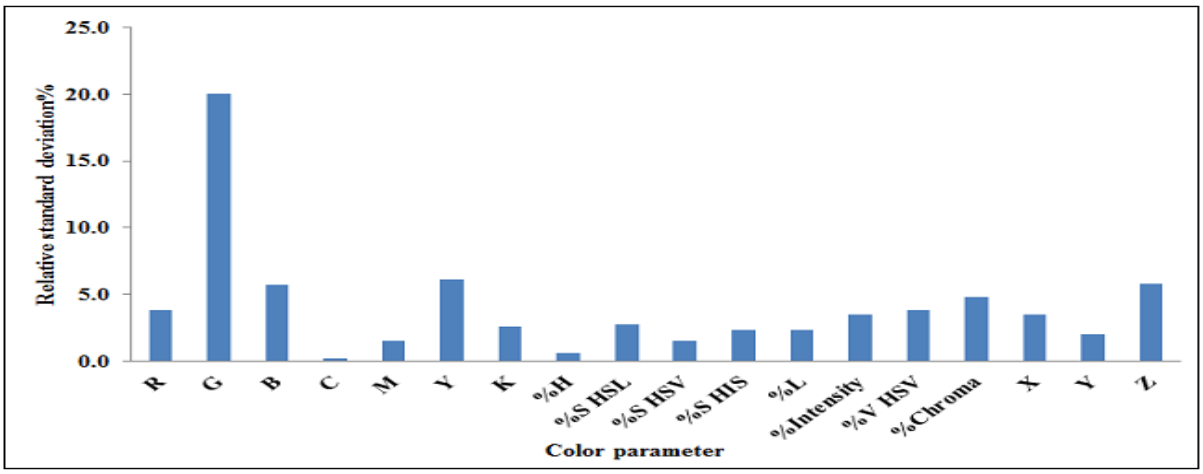

Fig. 8. Relative standard deviation on the color values for all color parameters at the various locations on glass plate for potassium permanganate 0.001 M, (conditions: sampling zone is central, format of images is jpg, resolution is 200 dpi).

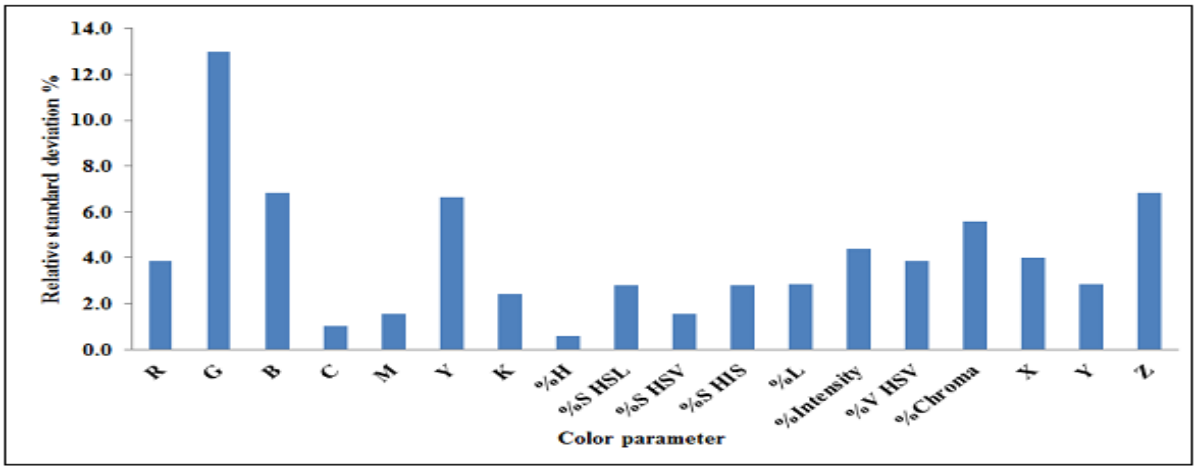

Fig. 9. Relative standard deviation of the color values for all color parameters at the various glass plate locations on the scanner surface for potassium permanganate 0.001 M, (conditions: sampling zone is central, droplet location on glass plate is central, format of images is jpg, resolution is 200 dpi).

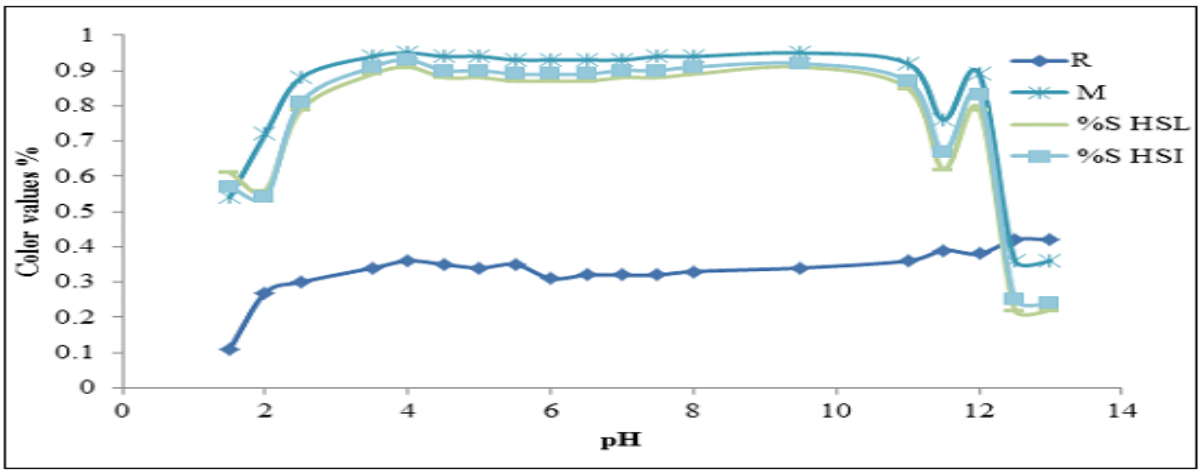

Fig. 10. Effect of pH on the color values of methyl violet, 0.01 M (conditions: resolution of scanner: 200 dpi, location of sampling box, droplet and glass plate: central, image format: jpg).

After appropriate time (20 minutes), five droplets of each solution were injected on the central location of glass plate. Image of droplets was transferred to color analyzing software by scanning with flatbed scanner. Fig.11 shows color values of Red, Magenta, $\%S_{HSL}$ and $\%S_{HSI}$ as representative of all color parameters. As results in the fig 11shows, the color values of cyanide-methyl violet product is independent of pH. Therefore in this study pH adjustment is not required.

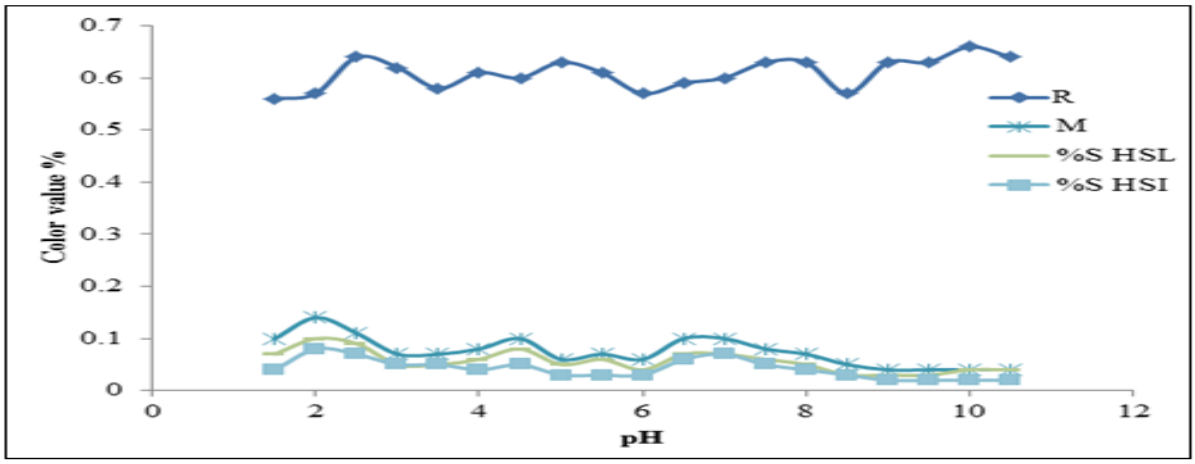

Fig. 11. Effect of pH on the color values of cyanide-methyl violet product, (conditions: methyl violet: 0.01 M, CN^-: 0.01 M, reaction time: 20 min, resolution of scanner: 200 dpi, location of sampling box, droplet and glass plate: central, image format: jpg).

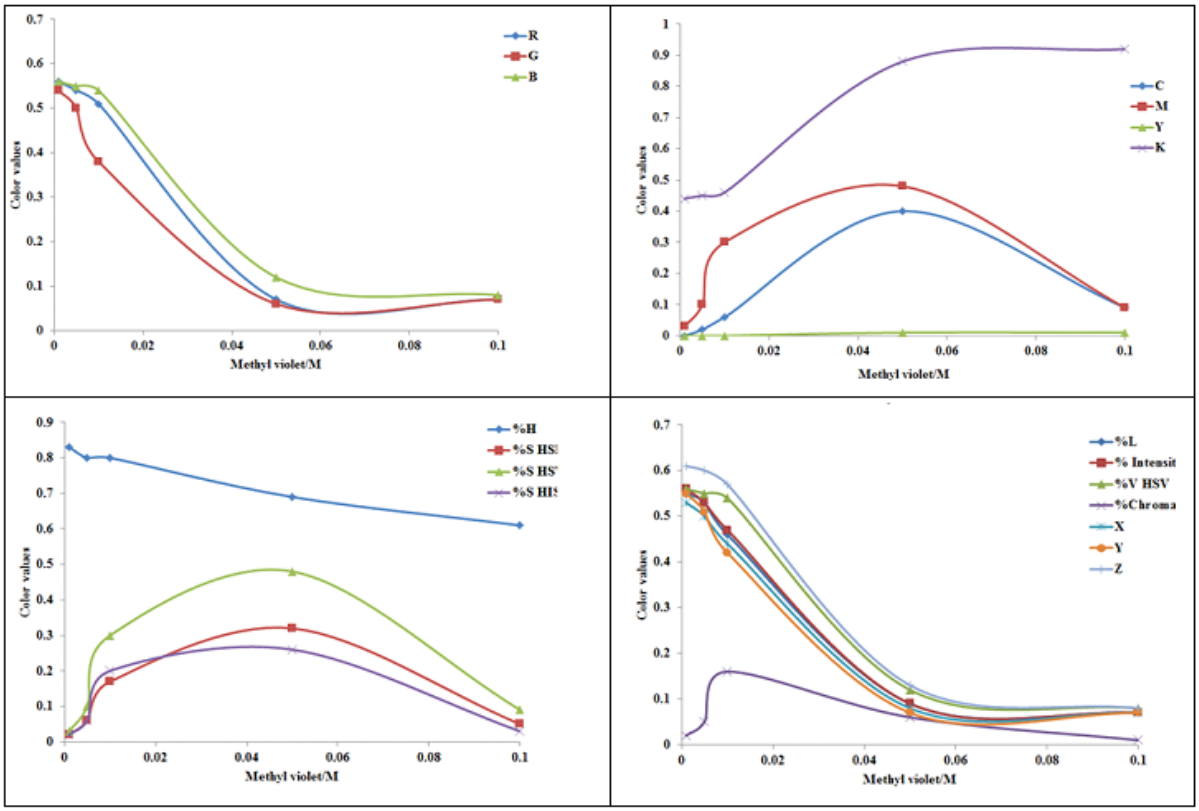

Fig. 12. Effect of methyl violet concentration on the color values of cyanide-methyl violet product in the various color models, (conditions: CN^-: 0.01 M, no pH adjustment, reaction time: 20 min, resolution of scanner: 200 dpi, location of sampling box, droplet and glass plate: central, image format: jpg).

Study of the effect of methyl violet concentration

To study the effect of methyl violet on the color values of cyanide-methyl violet product, solutions containing 0.01 M cyanide and various concentration of methyl violet were prepared. After appropriate time (20 minutes), five droplets of the product solution were injected on the glass plate and scanning was done. As results in the fig.12 show, for all color models, concentration of methyl violet of 0.05 M was selected as optimum for next experiments.

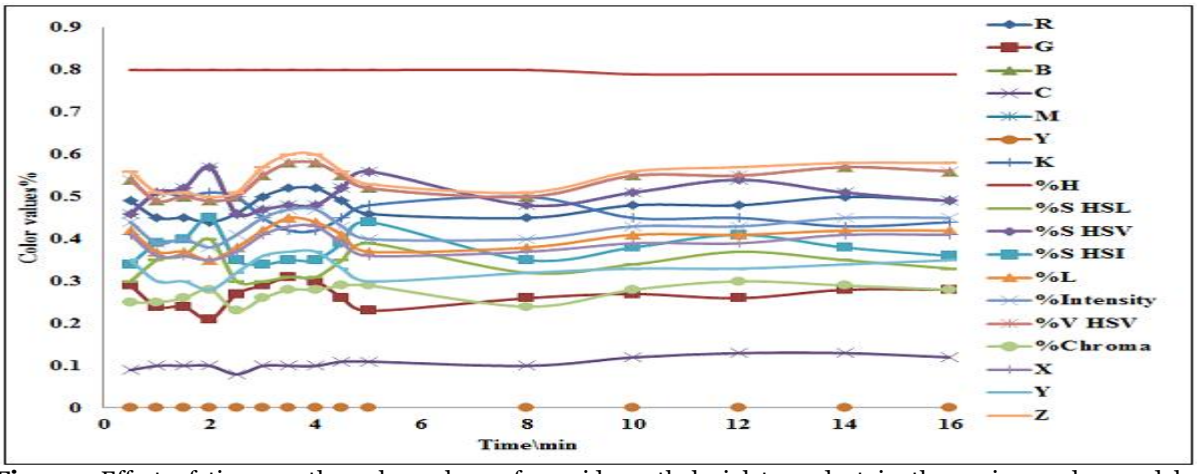

Fig. 13. Effect of time on the color values of cyanide-methyl violet product in the various color models, (conditions: CN^-: 0.01 M, methyl violet: 0.05 M, no pH adjustment, resolution of scanner: 200 dpi, location of sampling box, droplet and glass plate: central, image format: jpg).

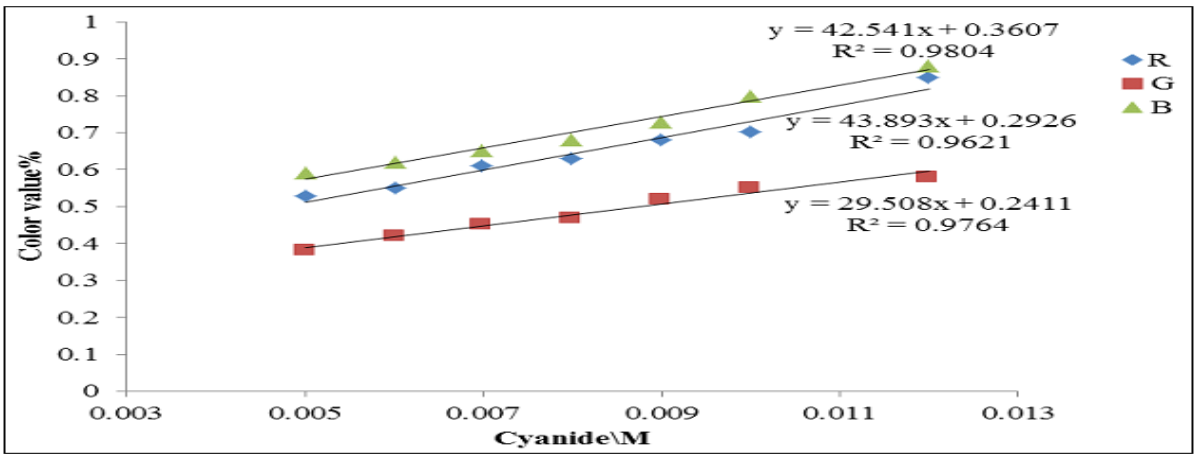

Fig. 14. Calibration curve in the RGB color system, under optimum condition.

Study the effect of times on the cyanide-methyl violet reaction

In order to study the effect of the time on the reaction between methyl violet and cyanide, solutions containing cyanide 0.01 M and methyl violet 0.05 M were prepared. After various times (1 – 16 minutes), five droplets of each solution were injected on the glass plate and scanning was done. Fig. 13 shows the results of this experiment. As the results show, before 10 minutes, irregular changes of color values depends on time were seen in the most color parameters. After 10 minutes, changes of color values were minimal. However, to ensure completeness of reaction time of 10 minutes was selected for the next experiments.

Calibration curve, linear range and detection limit

Under optimum conditions for all color parameters the calibration curves were plotted. Some color parameters show very bad linear range and linearity

(r^2< 0.6). Therefore fig. 14 to 17 show the calibration curves of the proposed method for some valuable color parameters. Linear range of 0.005 – 0.012 M cyanide (corresponding to 123.25 to 315 µg/mL) was obtained for all color parameters.

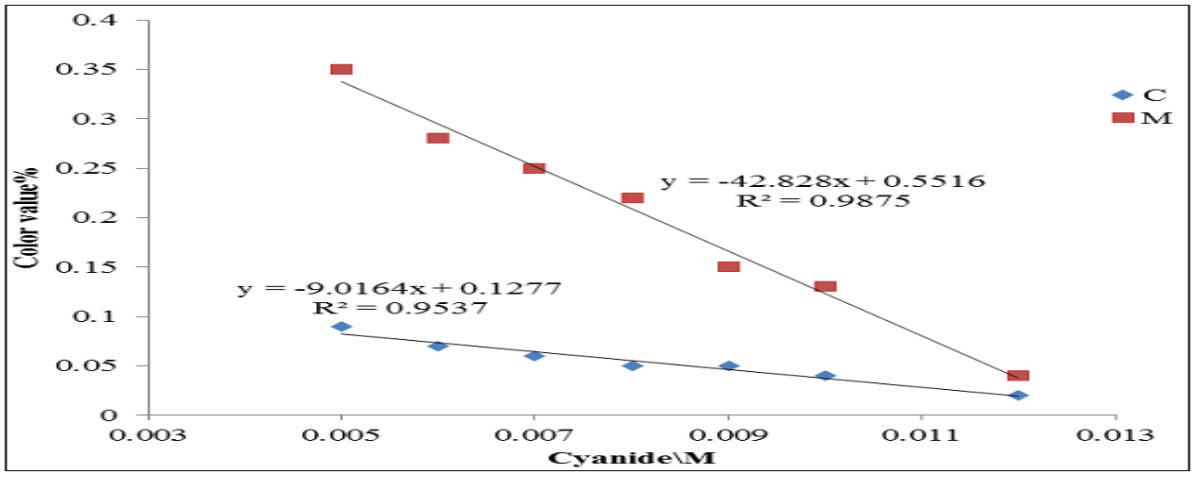

Fig. 15. Calibration curve in the CMYK color system (only for cyan and magenta), under optimum condition.

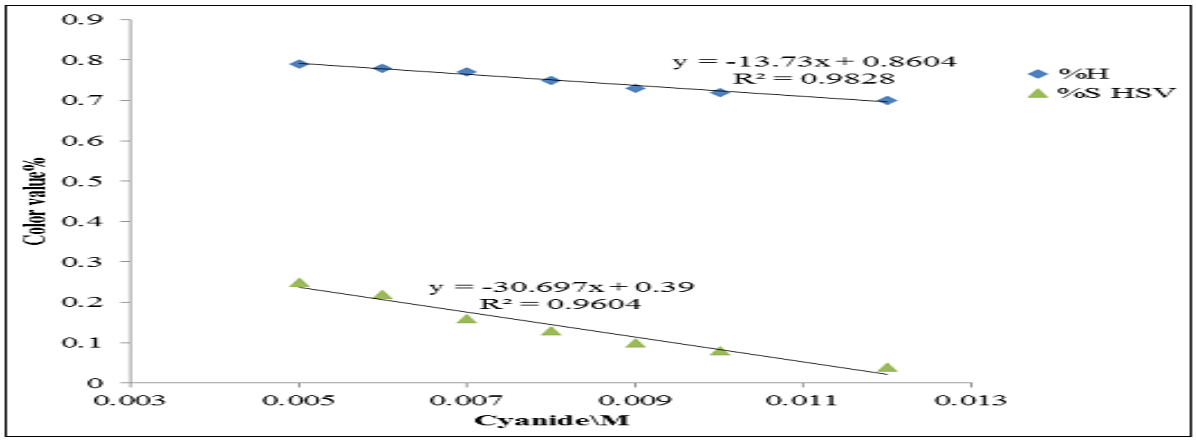

Fig. 16. Calibration curve in the HSV color system (only for H and S), under optimum condition.

For calculating of detection limit, a reagent blank was measured 10 times (n= 10) and a detection limit was obtained from three times of its standard deviation divided by the slope of the linear regression equation (LOD= 3SD $_{blank}$/m). For all color values, LODs were presented in the table 1.

According to the suggested procedure, under optimum conditions, 10 sample solutions of 0.006 M cyanide have been analyzed. Results for RSD of color parameters are presented in Table 2.

Study of interference

To study the selectivity of the developed method for the determination of cyanide, the effect of the presence of several species were investigated. Solutions containing 150 µg/mL cyanide and 500 µg/mL of interfering species were prepared and under the optimum conditions experiments were done. Table 3 shows the errors in the all color parameters for each interfering species. As illustrated in Table 3, no significant interference exists in all values for all interfering species except for $Cr_2O_7^{2-}$.

Application

The applicability of the method to assay cyanide in mineral water was examined. The amounts of

cyanide, which were spiked into the various brands of mineral water samples, were measured and relative errors are given for all color values in Table 4. From results in this table it was concluded that all color values are suitable for accurate determination of cyanide in real samples such as mineral waters.

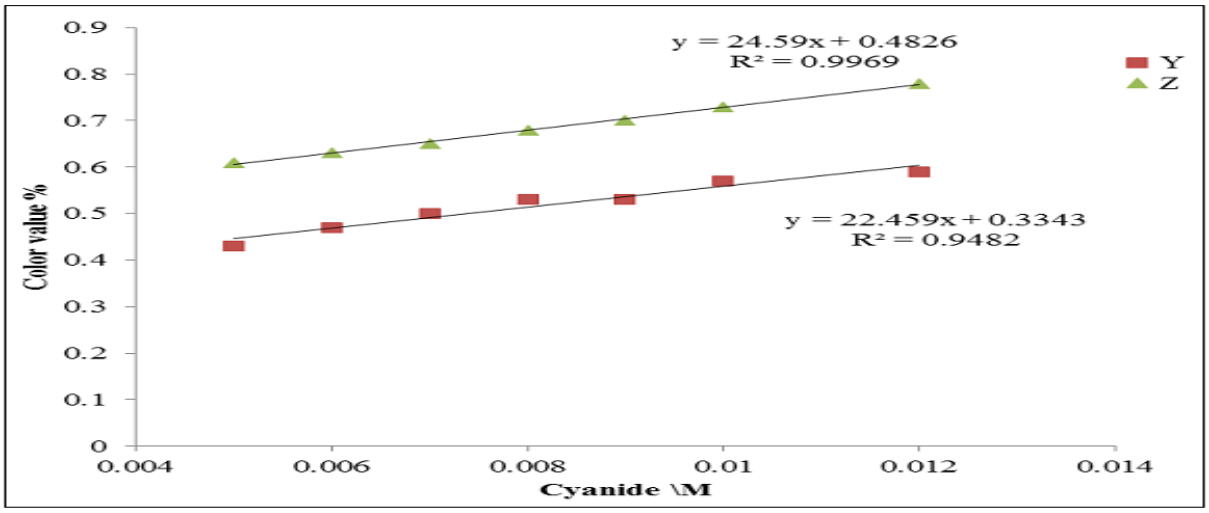

Fig. 17. Calibration curve in the XYZ color system (only for Y and Z), under optimum condition.

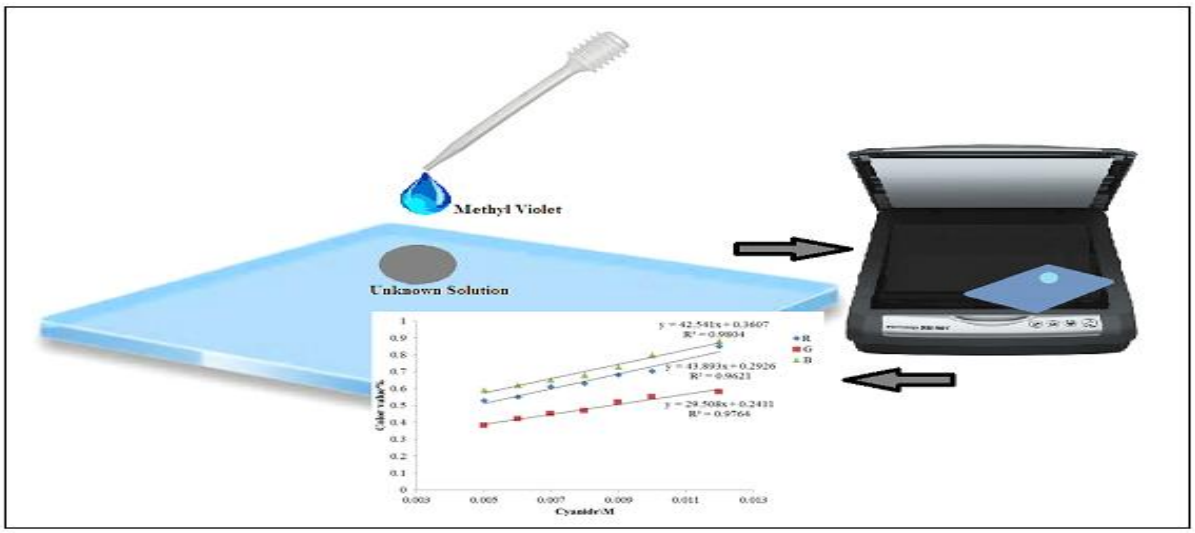

Fig 18. Graphical Index.

Conclusions

The single drop scanometry method described in this work is convenient for use in home and office. Also this method is simple, fast and inexpensive as an alternative to visible spectrophotometry.

Developed method only needs the scanner, a glass plate and a PC for analyzing the color. In this work, applicability of new method single drop scanometry was investigated for determination of cyanide in water samples. As results in table 5 show, this presented work have acceptable results(such as LOD and Linear range) relative to optical sensor (optode[11]) spectrophotometric method.

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