



Colorimetric Detection of Chromium using Laser Printed-Paper Based Microfluidics

1. Aim

To determine the concentration of chromium Cr (VI) sample through color intensity analysis.

2. Objectives

The proposed experiment has the following objectives:

- (i) Learn and apply the fundamentals of Beer-Lambert law
- (ii) Learn how to use ImageJ for image processing
- (iii) Determine the standard error and plot calibration curves
- (iv) Determine the concentration in an unknown sample, Limit of Detection (LOD), and Limit of Quantification (LOQ)

3. Theory

When light passes through a sample, certain spectrum of light may get absorbed or transmitted depending on the nature of the sample. The colour that we perceive is the light transmitted, complementary to the light absorbed. As amount of light absorbed increases, the light transmitted reduces and they are related by the formula

$$A = \log(I_0/I) = -\log(T) \quad (1)$$

Where A is absorbance, I is transmitted light intensity, I_0 is incident light intensity, T is transmittance. The light absorbed is related by Beer Lamberts Law which states that at dilute concentrations of analyte, the concentration varies linearly with absorbance of the solution, i.e. amount of light absorbed is directly proportional to concentration that particular solution.

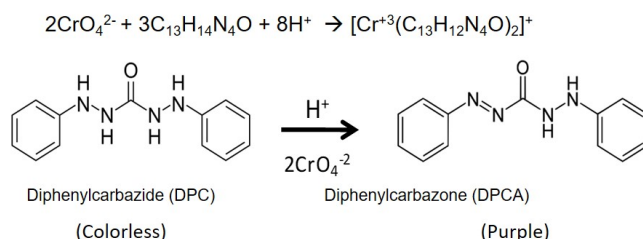
$$A = \epsilon cl \quad (2)$$

where ϵ is molar absorption coefficient (M⁻¹cm⁻¹), c depicts Molar concentration (M) and l denotes optical path length (cm). A calibration plot correlates known concentration of analyte with the intensity. This plot is used to determine the unknown concentration of the analyte.

Though for paper-based device, there is no optical path length, the intensity of the developed colour depends mainly on the thickness of the filter paper. For intensity measurement in paper-device, RGB analysis is used. Red, Green and Blue intensity ranges from 0-255. This analysis gives the intensity of light which is transmitted. The intensity value of the absorbed light decreases drastically with increase in concentration. For example, when purple colour is observed, green is absorbed and blue-red is transmitted by the body. RGB analysis indicates that the green (absorbed light) intensity value decreases with increase in purple (developed colour) colour, which results due to higher concentration of analyte. We plan to demonstrate the concept of colorimetric assay in paper-based microfluidics using chromium detection. The objective is to test whether the colorimetric assay performed in paper device follows Beer Lambert's Law.

For detecting chromium as spot assay, Diphenyl carbazide (DPC) method is used here. Oxidation of DPC with Cr(VI) ions in acidic environment yields DPCA-Cr(III) complex (Purple solution). This complex remains stable for 10 minutes after which Chromium(III) and DPCA degrades to respective compounds which leads to disappearance of the color with time.

The reaction that takes place is as follows:



Limit of detection (LOD) and Limit of Quantification (LOQ) is used to determine the sensitivity of the device. Limit of detection (LOD) is the lowest concentration of a substance that can be detected by an analytical method.

Limit of quantification(LOQ) is the lowest concentration of the substance that can be reliably measured with accuracy and precision. LOD and LOQ are determined by the following formula,

$$LOD = 3.3 * \frac{\sigma}{S} \tag{3}$$

$$LOQ = 10 * \frac{\sigma}{S} \tag{4}$$

Where σ is standard deviation of residuals (Excel steps = STEYX (Y-values, X-values)) S is slope of the regression line

4. Materials Required

Printed device provided by KA IPL, Diphenyl carbazide, 1000ppm Cr(VI) solution (prepared from $K_2Cr_2O_7$), Ethanol, 2N H_2SO_4 , Distilled water

5. Methodology

5.1 Analyte Preparation

- (i) Chromium stock solution of 1000 ppm is prepared initially.
- (ii) From this stock, 100 ppm chromium solution should be prepared.
- (iii) From this, working standard solution of 1,5,10,20,30,40 ppm are prepared.

5.2 Reagent preparation

- (i) 5 mg of DPC (sensitive to light) is dissolved in 5ml ethanol.
- (ii) Further 5ml of distilled water is added to the above mixture.
- (iii) Equal volume of this DPC mixture and 2N Sulphuric acid (say 0.2ml each) are mixed together.

5.3 Paper-based device

The paper device consists of 5 mm diameter circular hydrophilic region (6 spots in triplicate). The first spot is used as control and others for Cr (VI). Each experiments have to be triplicated.

5.4 Method

- (i) 2 μ L of colorimetric reagent is added on each spot and allowed to dry completely for 5 min.
- (ii) 5 μ L of chromium test solution is added on the circle and is let to stay 5 min for the solution to dry up leading to complete color development.
- (iii) For each concentration of chromium, three spots are used (experiment is repeated 3 times for better results)
- (iv) The experiment is performed for a blank solution(water), followed by 5,10,20,30,40 ppm of chromium solutions.
- (v) The image is captured at 5th minute using mobile camera/scanner (Figure 1)

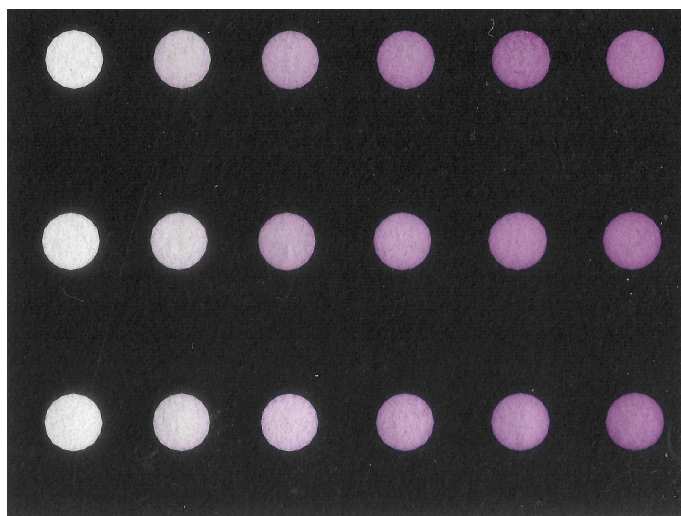


Figure 1: Chromium assay performed for different concentration (left most sample showing blank followed by 5,10,20,30,40ppm)

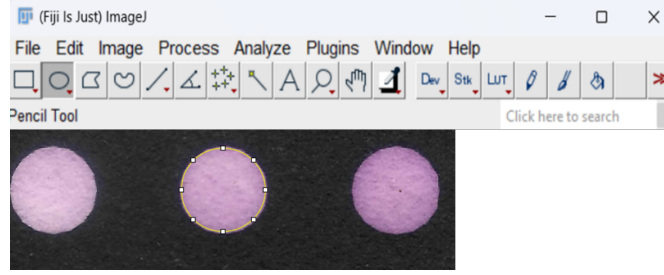


Figure 2: ImageJ (Fiji) software toolbar with captured image

6. Results and analysis

- (i) For color analysis, download and install ImageJ (Fiji) which is a free and open-source (shown in Fig2)
- (ii) Open the image in the software (File - Open)
- (iii) Draw the circle for the desired region of interest
- (iv) Obtain Green intensity (Analyze - Color Histogram)
- (v) Repeat the RGB analysis for known concentration (triplicates for all concentration) and unknown. Among these, Green intensity is more sensitive for chromium detection and is therefore used for plotting and calculation purposes. (Among mean, median and mode, mean is considered).

$$RelativeGreenIntensity = I_{gr0} - I_{gr} \quad (5)$$

Where I_{gr0} is intensity of blank solution and I_{gr} is the green intensity of a particular solution

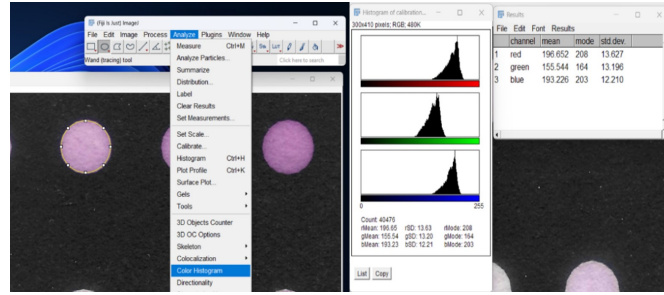


Figure 3: Image analysis step by step (analysis on left and results on the right)

- (vi) Plot a graph for known concentration of chromium (x-axis) and average of relative green intensity(y-axis) as shown in Fig 4. Plot trend line and generate equation for the plot. The unknown concentration can be estimated from the regression equation.
- (vii) To draw error bars, calculate standard deviation for each concentration separately (say 3 sample each) and divide it by square root of no of samples (in this case its sqrt (3)).
- (viii) From the graph, the values of limit of detection (LOD) and quantification (LOQ) are calculated.

ppm	I1	I2	I3	i1	i2	i3	Average	StDev	Error
0	148.762	147.642	142.77	0	0	0	0	0	0
5	90.543	99.159	85.54	58.219	48.483	57.23	54.644	5.358	3.09
10	82.629	78.764	72.587	66.133	68.878	70.183	68.398	2.067	1.19
20	73.606	36.621	36.376	75.156	111.021	106.394	97.523	19.508	1.26
30	39.425	33.404	20.055	109.337	114.238	122.715	115.43	6.768	3.90
40	8.93	8.925	8.923	139.832	138.717	133.847	137.465	3.182	1.83

Table 1: Sample data ranging from 5 – 40 ppm(Here I denotes green intensity and i denotes relative green intensity)

For the graph given below(Fig 4), Values obtained for this graph LOD = 4.46; LOQ = 14.89

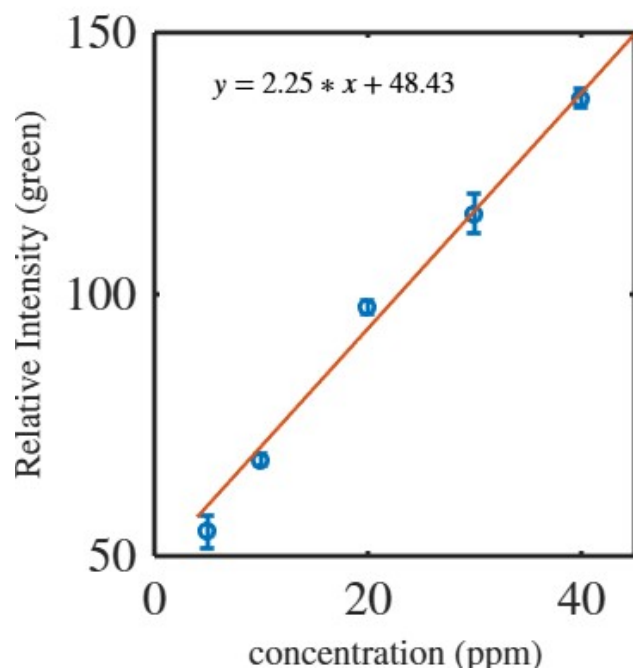


Figure 4: A typical excel graph plotted for Concentration vs Intensity.

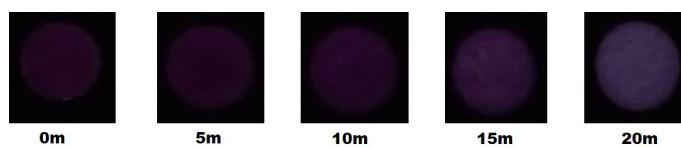


Figure 5: Change in intensity with time(0-20minutes) for 40ppm Cr(VI) solution

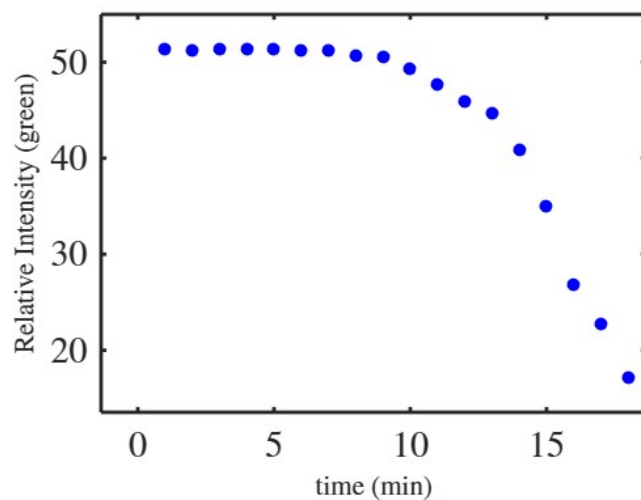


Figure 6: Change in green intensity with time for a 40ppm sample