COLORIMETRIC ANALYSIS OF ASPIRINE IN COMMERCIAL PHARMACEUTICALS

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Issues

1. Color systems and mechanism of their recognition
2. Basic terms of colorimetry (provided within this script)
3. Calculating concentrations and preparing dilutions

Introduction

Colorimetry

The word *colour* has double meaning: on the one hand it describes quality feature of an object, and on the other, esthetic impression of a viewer. The perceived colours are described by unique words, as blue, green, yellow, etc. These, in turn, cannot be defined using other words as they are considered as representation of fundamental concepts. Therefore, visual experience correlated with colours strongly depends on each individual observer making their perception as diversified as it can possibly be [1]. For that reason, it was necessary to introduce proper standards and procedures being able to represent coulour as numeric values; to make it happen, understanding how a human eye recognizes a colour is necessary.

The theories describing experience of an colour state, that its perception depends on three main factors:

1) Hue/tint, defined by wavelength of electromagnetic radiation (packages of photons) reflected from, or transmitted through an object.
   Human eye is able to detect electromagnetic radiation in range of 380-780 nm (visible light spectrum). If the colour of a non-transparent object is recognized as green, it means that its surface reflects wave light characterized by 500-560 nm wavelength, absorbing rest of the spectrum (Figure 1). On the other hand, if the same colour is observed in case of a transparent object, it means that it transmits the same wave light, absorbing rest of the spectrum.

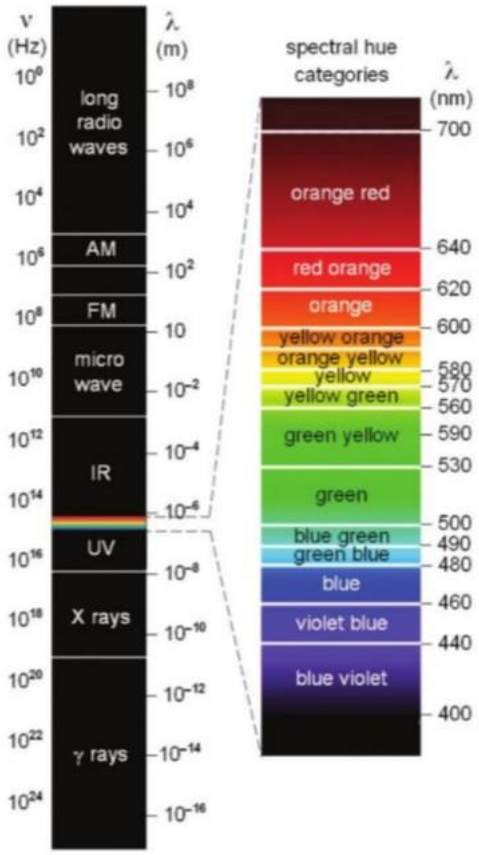

Figure 1. Spectrum of electromagnetic radiation characterized as wavelength [1]

2) **Chroma/Saturation**, defining if a color is monochromatic, or, using other words, how vivid or faded a colour is. Monochromatic ones are the most saturated, the most vivid; non-monochromatic in turn, are close to gray, being faded.

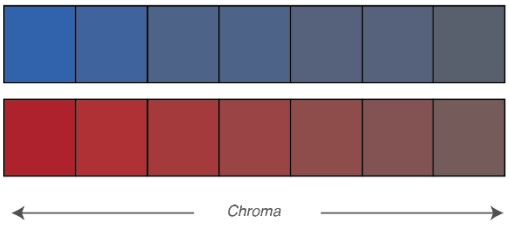

Figure 2. Chroma of blue and red colours [2]

3) **Brightness** defined by amount of the reflected/transmitted light; determines experience correlated with dark or bright colours

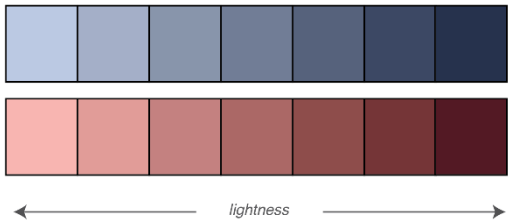

Figure 3. Brightness of blue and red colours [3]

Moreover, experience of a colour is also dependent on variables not directly linked to hue, saturation and brightness. These are:

1) **degree**, at which a virtual observer is experiencing a colour (value called *standard observer*)
2) **type of the light** illuminating an observed object; we can distinguish the following types of illuminants [4]:
    a. **A**, domestic light induced by tungsten filament with correlated colour temperature (CCT) of 2856K
    b. **B,** daylight, CCT=4874K
    c. **C,** daylight, CCT=6774K
    d. **D,** daylight at variable CCT
    e. **E,** illuminant with controlled distribution of the visible spectrum, gives equal energy for all wavelengths
    f. **F,** fluorescent illuminants

Because humans' perception of a colour is dependent on the mentioned above variables, a final model describing its parameters must be 3-Dimensional, which complicates the whole matter. Common colour systems, used for instance in computer displays (RGB- *Red Green Blue*) or printing devices (CMYK-*Cyan Magenta Yellow Black*) are very important from practical point of view, however they are not sufficient as precise description of an colour, expressed in 3D system [1]. One of the first systems taking into account 3D position of an colour as a function of hue, saturation and brightness was introduced by Munsell in 1900s [4]

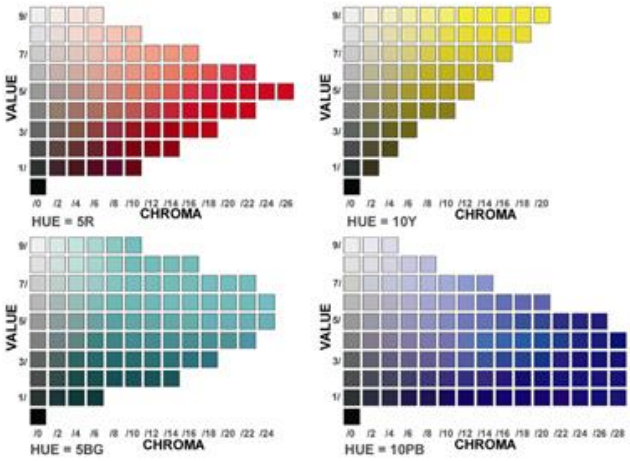

Figure 4. An example of Munsell system for four different hues [4]

Despite its advance at the time, the main practical disadvantage of the Munsell colour system is that the colours are defined only for one *standard observer* (2°) and only for one type of light illuminating an object (C). For that reason it was necessary to develop more advanced model.

Nowadays the most popular colour system, used in various types of industries is CIE *L*a*b space created by International Commision on Illumination. This system is reflecting how a human's eye recognizes differences between colours and assumes, that difference between them is linear, opening the path for colorimetric analysis.

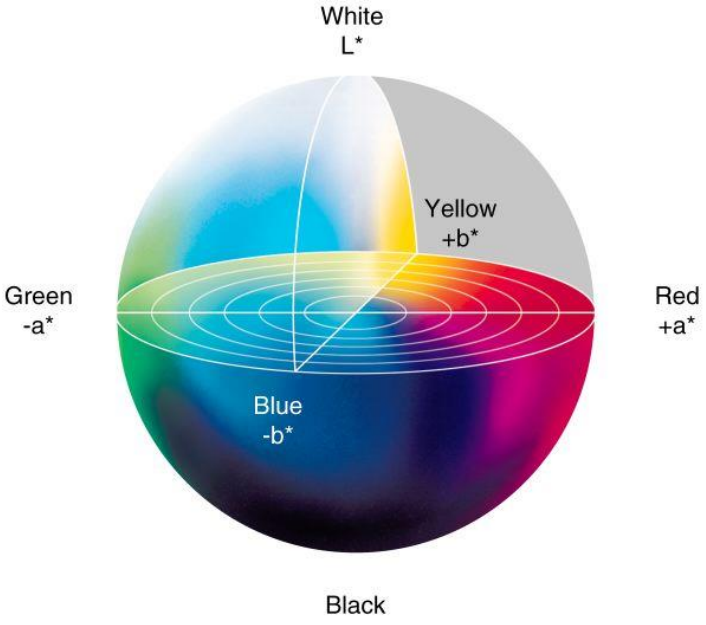

Figure 5. CIE *L*a*b space [5]

The system CIE *L*a*b is defined by three parameters:

1) L: light versus dark, the lower number is, the darker colour is observed

2) **a**: red versus green, where positive values means red, negative, in turn, green
3) **b**: yellow versus blue, where positive values means yellow, negative, blue

The *L*a*b parameters are linked to each other by cube root function, which means, that creating an analytical curve in this 3D system (Figure 5) would require usage of complicated equations and specific software able to create visual representation of the collected data. However, the major advantage of CIE *L*a*b system is the possibility of determination of difference between colors expressed as numeric value ΔE. This parameter is defined as:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (1)$$

Because of the fact, that ΔE changes proportionally with actual change of the observed colors it is possible to transfer *L*a*b parameters into 2-dimensional system expressed as ΔE=f(C), where color is a function of concentration (C).

Colorimetric analysis of aspirin

The objective of the present exercise is to apply colorimetric procedure, using CIE *L*a*b space to determine concentration of aspirin in samples of commercial pharmaceuticals.

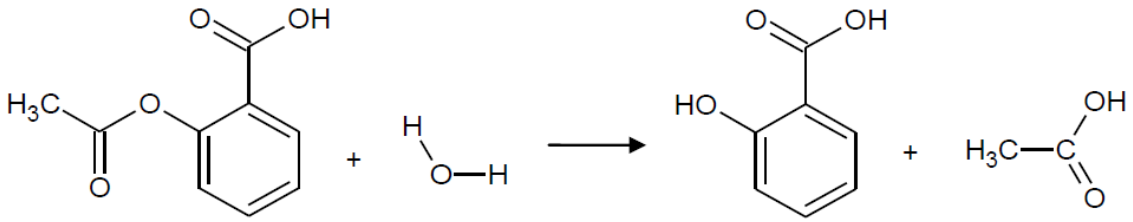

Figure 6. Hydrolysis of acetylsalicylic acid

Acetylsalicylic acid hydrolyses to 2-hydroxybenzoic acid. This, in turn, creates stable, violet-blue complex with Fe(III) ions becoming red with decreasing amount of aspirin. Based on that phenomenon, concentration of hydrolyzed aspirin can be determined by defying the intensity of the color complex. From this value, amount of the active agent in commercial pharmaceuticals will be calculated.

[1] C. Oleari, *Standard Colorimetry: Definitions, Algorithms and Software,* John Wiley&Sons, Chichester, *2016*

[2] website: http://blogs.adobe.com/creativecloud/files/2014/06/TenWays-chroma.png, access date: July 14th, 2016

[3] website: http://blogs.adobe.com/creativecloud/files/2014/06/TenWays-lightness.png, access date: July 14th, 2016

[4] D. Malacara, *Color Vision and Colorimetry: Theory and Applications, Second Edition,* SPIE Press, Bellingham*, 2002*

[5] website: http://www.coatsindustrial.com/pl/images/Colour%20difference_tcm81-152855.jpg, access date: July 14th, 2016

[6] R.F. Witzel, R.W. Burnham, J.W. Onley, *Threshold and suprathreshold perceptual color differences,* J. Optical Society of America 63, 615-625, 1973

Procedure of analysis

Equipment and materials

- CIE *L*a*b colorimeter equipped with proper measuring head
- 100 cm^3 Erlenmeyer's (conical) flasks
- Electric heater
- Measuring cylinder
- Funnel+filter
- Beakers
- 500 cm^3 volumetric flasks
- 100 cm^3 volumetric flasks
- Automatic pipette
- Aspirin, ACS Reagent, 99%
- 1 $mol \cdot dm^{-3}$ sodium hydroxide solution
- 0.02 $mol \cdot dm^{-3}$ iron(III) chloride solution
- Commercial samples

Preparing blank

1. Add 50 cm^3 of 1.0 $mol \cdot dm^{-3}$ sodium hydroxide solution into 250 cm^3 conical flask and warm it at 50 °C for 10 minutes
2. Cool the solution and transfer quantitatively to a 500 cm^3 volumetric flask. Make up to the mark with deionized water.
3. Use a pipette to measure 5 cm^3 of the solution prepared in the point 2 into 100 cm^3 volumetric flask. Make it up to volume with 0.02 $mol \cdot dm^{-3}$ iron(III) chloride solution. This is the **blank**
4. Measure and note the parameters *L*a*b of the blank solution using the colorimeter, fitted with a suitable measuring head.

Preparing standard solutions

1. Weigh accurately about 0.4 g of aspirin into a 250 cm^3 conical flask, using cylinder add 50 cm^3 of 1.0 $mol \cdot dm^{-3}$ sodium hydroxide solution and warm the mixture gently at 50 °C for 10 minutes.
2. Cool the solution and transfer quantitatively to a 500 cm^3 volumetric flask. Make up to the mark with deionized water. This is the stock solution. It contains the hydrolysis product (sodium 2-hydroxybenzoate) from a 0.80 $g \cdot dm^{-3}$ solution of aspirin.
3. Use an automatic pipette to measure 5 cm^3 of stock solution into a 100 cm^3 volumetric flask. Then, fill the flask up using 0.02 $mol \cdot dm^{-3}$ iron(III) chloride solution. This is standard solution A. In a similar way make up standard solutions B to G using 4 cm^3, 3 cm^3, 2 cm^3, 1 cm^3, 0.5 cm^3 and 0.25 cm^3 of the stock solution, respectively.
4. Wait approximately 15 min to allow the color to be fully developed.
5. Measure and note the difference of *L*a*b parameters of each standard solution against white background using the colorimeter fitted with a suitable measuring head. Repeat each measurement 3 times to make an average from the results.

6. Calculate parameter ΔE of each standard solution and draw a calibration curve as a function ΔE=f(C). Select sufficient measuring range for the method.

Analyzing aspirin tablets

1. Make a note of the mass of aspirin the manufacturer states is in each tablet. This is given on the packaging.
2. Consider a potential concentration of the solution you are going to receive. Determine suitable dilutions you must make.
3. Transfer a tablet into a 250 cm^3 conical flask. Carefully crush the tablet with a stirring rod and add 50 cm^3 of 1 $mol \cdot dm^{-3}$ sodium hydroxide solution and warm it gently at 50 °C gently for ten minutes.
4. Cool the solution and transfer quantitatively to a 500 cm^3 volumetric flask, filter any insoluble material if necessary. Make up the flask to the mark with deionized water.
5. Based on the predicted concentrations add an appropriate amount of the solution prepared in the point **3** into a 100 cm^3 volumetric flask using automatic pipette. Then, fill the flask to the line using 0.02 $mol \cdot dm^{-3}$ iron(III) chloride solution.
6. Wait approximately 15 min to allow the color to be fully developed.
7. Measure the *L*a*b parameters of the unknown against white background using the colorimeter fitted with a proper measuring head

Analysis of the results

Calibration curve

Mass of 99% Aspirin: ..... g

Volume of the stock solution: ..... dm^3

Concentration of aspirin in the stock solution: ..... $g \cdot dm^{-3}$

Solution	V_s[1]	V_T[2]	C[3]	*L[4]	*a[4]	*b[4]	ΔE[5]
blank							
				Δ*L	Δ*a	Δ*b	
A							
B							
C		100					
D							
E							
F							
G							

[1] Taken volume of the stock solution [cm^3], [2] Target volume [cm^3], [3] Concentration of aspirin [$g \cdot dm^{-3}$], [4] Average values of CIE parameters, [5] difference of colour (Eq.1) between a solution A-G and the blank

Plot the calculated ΔE_{A-G} against concentration C. Then, approximate the curve using linear function; use the obtained equation for further procedures.

Analysis of aspirin in the prepared samples

Producer	m_d[1]	C_{500}[2]	V_T[3]	D[4]	Δ*L[5]	Δ*a[5]	Δ*b[5]	ΔE[6]	$m_{aspirin}$[7]

[1] declared mass of aspirin [g], [2] Predicted concentration in 500 cm^3 flask, [3] Volume transferred into 100 cm^3 flask, [4] Applied dilution, [5]Average values of the CIE parameters, [6] difference of a colour (Eq.1) between a solution and the blank, [7] Calculated mass of aspirin in a tablet

Use the calibration curve to calculate amount of aspirin in 100 cm^3 volumetric flask. Then, **taking into account all of the dilutions made during the procedure**, recalculate this value to receive mass of the analyzed aspirin.

Report

The report should contain:

1. Name and index number
2. Brief description of the carried out analyses
3. Filled tables from the pages 8 and 9
4. Calibration curve
5. Examples of calculations
6. Conclusions