APPLICATION NOTE

ASSESSING THE COLOR AND BITTERNESS OF BEER ACCORDING TO THE SRM AND EBC METHODS USING BOTH A CUVETTE AND A FLOW CELL

INTRODUCTION

Beer is an extremely popular alcoholic beverage with a vast and strong market all over the world. In recent years, craft breweries are growing in number and total volume sold [1]. Owing to the increase of craft beers, drinkers are starting to learn more about the different beer styles, namely their distinctive colors and flavors [1,2].

The quality of beers can be investigated through different analytical methods. Among the 12 inspection items proposed by the American Society of Brewing Chemists (ASBC), color and bitterness can be evaluated using UV-Vis spectrophotometry. These inspection items are in line with the standard methods for color and bitterness evaluation by the European Brewery Convention (EBC) and Institute of Brewing and Distilling (IBD) [3-8].

Beer has a wide range of colors, which are evaluated according to a spectroscopic procedure. Beer color is an important parameter for quality control during the brewing process [9].

Keywords:

UV-Vis Spectroscopy;

DWHP Light Source;

FLEX Spectrometer;

Beer Color Analysis;

Beer Bitterness Analysis;

Absorbance Cuvette vs Flow Cells

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The Standard Reference Method (or SRM) is one of several methods specified by the ASBC and EBC used extensively by brewers for beer color assessment (**Figure 1**) [3,4]. In addition to the SRM method, EBC also specifies the EBC method [4]. These methods are quantitative and involves the absorbance measuremen of a turbidity-free beer sample [3,4].

Figure 1 – EBC and SRM beer color grade.

The standard method for measuring the bitterness of beers requires the extraction of iso-alpha acids from the acidified beer (beers are acidified because yeast grows better in slightly acidic pH). Changes in pH can also be used to alter the flavor profile of the final beer using iso-octane [10,11].

Iso-alpha acids, which are the main source of bitterness in beer, are produced from the alpha acids when hops are boiled [11]. Following the extraction of the iso-alpha acids, the bitterness units (BU) are determined by measuring the absorption of the iso-octane layer.

In this application note, we combined a high-power deuterium and tungsten-halogen (DWHP) light source with a FLEX spectrometer to measure the SRM and EBC color grade, and bitterness units (BU) of four different commercially-available styles of beer according to the ASBC and EBC methods.

In order to evaluate the accuracy and flexibility of Sarspec's absorbance configuration, SRM and EBC color grade were measured using a standard quartz cuvette and flow injection cell.

MATERIALS & METHODS

Reagents

· Four different styles of commercially-available beers (Dark Lager, IPA, Japanese Lager, and Munich Weiss);
· Distilled Water;
· Octanol;
· Iso-Octane;
· Hydrochloric Acid 3M;

Instruments and Accessories

· DWHP Light Source;
· Two Optical Fibers with 200 µm of diameter;
· Standard Cuvette Holder set into an absorbance configuration;
· FLEX Spectrometer (Slit: 10 µm; Grating: 600 grooves/300 nm; Detector: Sony 2048 pixel; Improved Sensitivity);
· 10 mm and 1 mm Quartz Absorption Cuvettes;
· 3 mm Absorbance Flow Injection Cell;

Figure 2 – Sarspec's light source (DWHP), spectrometer (FLEX), and accessories (Standard Cuvette Holder and Absorbance Flow Injection Cell). All these instruments were connected using optical fibers with a diameter of 200 µm.

EXPERIMENTAL PROCEDURE

Procedure for measuring beer color (with Standard Cuvette Holder)

1. Introduce distilled water into a quartz cuvette and use it as reference;

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2. Introduce a degassed beer sample (beer samples needs to be degassed in order to avoid unreliable measurements as a result of a constant flow of air passing through the optical path) into a second quartz cuvette. For clear beer samples, such as Munich Weiss and Japanese Lager, a 10 mm quartz cuvette was used; while darker beer samples, such as IPA and Dark Lager, owing to its large absorbance values around 430 nm, the 1 mm quartz cuvette was used. The absorbance was measured at 430 nm and 700 nm (the absorbance at 700 nm allows to evaluate the turbidity of the beer sample). The instrument settings were as in **Table 1**;

3. If the beer sample has an absorbance value with the 10 mm quartz cuvette higher than 1.0, or is not free of turbidity (the absorbance value at 700 nm is lower than the value obtained by multiplying the absorbance value at 430 nm by 0.05), repeat the measurement with a 1 mm quartz cuvette or after diluting it;

4. Calculate the SRM color grade using the following equation:

$$SRM=10\times d\times p\times A_{430\ nm} \quad (1),$$

where d is the dilution factor, p is the optical path conversion factor (1.27 if using 10 mm cuvettes), and $A_{430\ nm}$ is the absorbance value of the beer sample at 430 nm (the absorbance should be divided by 0,1 when measurements are performed with 1 mm quartz cuvettes to correct the absorbance to a standard 10 mm optical path). The EBC color unit can be calculated from the SRM scale by using the following equation [4]

$$EBC\ unit=SRM\ unit\times 1.97. \quad (2)$$

Table 1 – Instrument settings used for the experimental measurements with the standard cuvette holder.

Parameter	Value
Integration time (ms)	2
Average	150
Smoothing	6

Procedure for measuring beer color (with Absorbance Flow Injection Cell)

1. Introduce distilled water into a small container with the circulation pump connected to the absorbance flow injection cell;

2. Turn the circulation pump on and perform the reference and dark measurements with distilled water;

3. After performing reference and dark measurements, carefully remove the distilled water from the small container and add the degassed beer sample (after turning the circulation pump on with the beer sample, avoid to dilute it with the distilled water inside the flow injection cell);

4. Measure the absorbance at 430 nm and 700 nm (the absorbance at 430 nm should be divided by 0.3 for measurements performed with the flow cell to correct the absorbance to a standard 10 mm optical path). The instrument settings used in this configuration are in **Table 2**;

5. Calculate the SRM and EBC color grade using *Eq. 1* and *Eq. 2*, respectively;

Table 2 – Instrument settings used for the experimental measurements with the flow injection cell.

Parameter	Value
Integration time (ms)	35
Average	100
Smoothing	6

Procedure for measuring beer bitterness (indirect measurement that requires only the Standard Cuvette Holder)

1. Introduce 10 mL of a degassed beer sample into a 50 mL conical flask;

2. Add 50 µL of octanol;

3. Add 1 mL of 3 M hydrochloric acid and 20 mL of iso-octane;

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4. Shake the mixture until an emulsion is formed;

5. Add the emulsion into a centrifuge tube;

6. Centrifuge the emulsion for 3 min, at a minimum of 3000 rpm, to separate the aqueous layer from the organic layer;

7. Add iso-octane into a 10 mm cuvette and use it as reference;

8. Add the organic layer of the mixture into a 10 mm quartz cuvette and measure the absorbance at 275 nm. The instrument settings used for this experiment are displayed in **Table 3**;

9. Calculate the BU using the following equation:

$$BU = 50 \times A_{275\ nm} \quad (3),$$

where $A_{275\ nm}$ is the absorbance value of the mixture at 275 nm in a 10 mm quartz cuvette.

Table 3 – Instrument settings used for the experimental measurements with the standard cuvette holder.

Parameter	Value
Integration time (ms)	120
Average	50
Smoothing	3

RESULTS

Beer Color

The absorbance of each beer sample was measured between 400 and 700 nm using a configuration with the standard cuvette holder. The absorbance values for each beer sample at 430 and 700 nm, and the SRM and EBC values obtained according to Eq. 1 and Eq. 2, respectively, are given in **Table 4**.

Table 4 – Results of color measurements of beer samples using standard cuvette holder (SCH).

Beer Sample	Absorbance @ 430 nm	Absorbance @ 700 nm	Optical Pathlenth	SRM Value	EBC Value
1 (D. Lager)	0.275	0.010	1 mm	35	69
2 (IPA)	0.134 / 1.36	0.009 / 0.008	1 mm / 10 mm	17 / 17	34 / 34
3 (Jap. lager)	0.329	0.004	10 mm	4	8
4 (M. Weiss)	0.504	0.025	10 mm	6	13

The results in **Table 4** show that the SRM and EBC values are lower for the Japanese Lager (beer sample 3) and higher for the Dark Lager (beer sample 1). On the other hand, turbidity is higher for Munich Weiss (beer sample 4).

In order to demonstrate the large flexibility of our absorption configuration, the SRM and EBC values of each beer sample were calculated using a completely different setup, with an absorbance flow cell. The liquid beer set in the container with a circulation pump would go through the 3 mm optical path absorption cell in a continuous flow (the absorbance values obtained with a 3 mm flow cell were corrected according to what was stated experimental in the procedure section).

The absorbance at 430 and 700 nm, as well as the SRM and EBC values for the configuration with the flow cell are in **Table 5**.

Table 5 – Results of color measurements of beer samples using an absorbance flow cell (FC, 3 mm optical path).

Beer Sample	Absorbance @ 430 nm	Absorbance @ 700 nm	SRM (FC)	SRM (SCH)	EBC (FC)	EBC (SCH)
1 (D. Lager)	0.774	0.041	33	35	65	69
2 (IPA)	0.374	0.012	16	17	32	34
3 (Jap. lager)	0.109	0.002	5	4	9	8
4 (M. Weiss)	0.146	0.011	6	6	12	13

The SRM and EBC values in **Table 5** are very similar to those in **Table 4**. Minor differences can be attributed to the degassing procedure.

In addition to single wavelength measurements (at 430 nm), the color of the beer samples was also assessed using the tristimulus method, a feature that is integrated in LightScan software. After measuring the transmission spectra of each sample in the whole visible wavelength range, it is possible to define the L*, a*, b* color space parameters that are defined in the CIE standard colorimetric system, **Table 6** [9].

This method makes it possible to acquire color-related information that cannot be obtained by the single-point method.

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Table 6 – Color measured for each beer sample in the standard cuvette holder (with a 10 mm quartz cuvette) using the CIE standard colorimetric system.

Beer Sample	L*	a*	b*	Color
1 (Dark Lager)	56.2	27.3	78.7	
2 (IPA)	73.7	12.0	61.2	
3 (Japanese lager)	91.7	0.77	28.3	
4 (Munich Weiss)	83.9	3.07	37.1	

The color obtained from the CIE coordinates with the LightScan software (**Table 6**) follows the trend observed for the SRM and EBC values displayed in **Tables 4 and 5**. As the color of beer becomes brighter, the SRM and EBC values decrease.

Bitterness of Beer

The absorbance values obtained at 275 nm, and the results of the BU values calculated using *Eq. 3* for the beer samples are in **Table 7**.

Table 7 – Results of bitterness determined in the beer samples using standard cuvette holder.

Beer Sample	Absorbance @ 275 nm	Bitterness Units (BU)	Bitterness Range [Ref.]
1 (Dark Lager)	0.37	18	15 – 25 [12]
2 (IPA)	1.02	51	40 – 60 [12]
3 (Jap. lager)	0.32	16	5 – 20 [13]
4 (Mun. Weiss)	0.20	10	10 – 15 [12]

According to the results (**Table 7**), beer 2 (IPA) has the highest BU value, while beer 4 (Munich Weiss) has the lowest BU value. Both beers 1 and 3 have quite similar BU values. The obtained BU values for each beer sample shows to be within the bitterness range published for each beer style [12,13].

CONCLUSIONS

An absorption configuration with different experimental approaches (cuvette and flow cell) was used in this application note to determine the color and bitterness of four different beer samples.

The color of each beer sample was assessed using the absorbance values, which allowed the determination of both the SRM and EBC values, and the tristimulus method, which allowed the determination of the visual color through CIE coordinates.

The bitterness unit, on the other hand, was determined through the absorbance values, at 275 nm. Each determined value was within the estimated bitterness range for the respective beer style.

This application note highlights the great accuracy and large flexibility of Sarspec spectrometers and accessories in absorbance measurements of solutions in different situations. By using both a cuvette holder and a flow cell, we were able to demonstrate that color can be quickly and accurately measured for solutions either in small samples (quartz cuvettes) or in large containers (it can be set into a continuous circulation through a flow cell).

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