UV-Vis Spectrophotometer Comparison

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Abstract

UV-Vis spectrophotometers are used routinely to determine the concentration of solution species from the measured absorbance of light in accordance with Beer's Law. A study was undertaken to compare the quantitative performance of five different spectrophotometers and one colorimeter that have been recently acquired by the chemistry department. Knowledge of this information will aid in selecting between these instruments for different applications. Figures of merit were obtained for the determination of iron (III) concentrations using thiocyanate ion to produce a red colored complex. Standards were prepared from a 995 ppm iron(III) standard and analyzed simultaneously on each instrument. Absorbance was measured at 479 nm for each standard. All five spectrophotometers gave analytical sensitivities of 0.172±0.004 L/mg. As expected, the colorimeter had a lower analytical sensitivity, 0.117 L/mg, due to the use of broadband LED light source. The best linear dynamic range was observed for the Cary 300 research grade spectrophotometer: 0.005-24 ppm. The poorest limit of quantitation (low limit), 0.63 ppm, observed for the Spectronic 200. The poorest limit of linearity (upper limit) was 8 ppm (Vernier UV-Vis). Analytical sensitivities, detection limits and wavelength ranges were also compared.

Introduction

The comparison of figures of merit is a useful approach for the selection of an instrument for a given experiment[1]. The choice of instrument depends on the purpose of the experiment. For example, qualitative analysis would require a spectrometer that can scan a range of UV-Vis wavelengths, whereas for quantitative analysis the spectrophotometer must be able to detect light levels precisely. Figures of merit are quantitative characteristics that are used to evaluate the suitability of a device or instrument. Calibration sensitivity, analytical sensitivity, detection limit and linear dynamic range are commonly used to compare instruments used for quantitative analysis[1].

Spectrophotometry is an analytical method that measures how strongly a sample absorbs electromagnetic radiation and uses that information to determine the concentration of a substance. UV-Vis spectrophotometry is one of the most widely used methods of quantitative analysis in chemistry, physics, biology and biochemistry. Visible spectrophotometers cover the visible region of the spectrum from ca. 400-700 nm.

UV-Vis spectrophotometers include UV wavelengths from ca. 180-400 nm. Spectrophotometers are routinely used to measure the fraction of light absorbed at a given wavelength, measured as absorbance (A) which is directly proportional to the concentration of an analyte as described by Beer's Law: $A = \varepsilon bc$

There is a great deal of variation in spectrophotometer design and capabilities[2]. The specifications of the six spectrophotometers examined in this study are listed in Table 1.

Instrument	Scanning Feature	Optical Design	Detector	Light Source	Wavelength Range	Manufactured by	Price
Cary 300 [3]	Yes	Double, single, dual beam	Photomultiplier tube	Deuterium & Tungsten	190-900nm	Agilent	$34,000
GENESYS 20 [4]	No	Single beam	Silicon Photodiode	Tungsten-Halogen	325-1100nm	ThermoScientific	$2,212
Spectronic 200 [5]	Yes	Single beam	CCD	Tungsten-Halogen	340-1000nm	ThermoScientific	$2,095
Vernier UV-Vis [6] Spectrophotometer	Yes	Single beam	CCD	Deuterium & Incandescent	220-850nm	Vernier	$1,999
Colorimeter [7]	No	Single beam	Photodiode	LED	430nm, 470nm 565nm, 635nm	Vernier	$115**
Red Tide [8]	Yes	Single beams	CCD	Tungsten & Deuterium	200-850nm	Vernier/Ocean Optics	$3,349

Table 1- Features of UV-Vis spectrophotometers in the ASU Chemistry Department

Method

The instruments were warmed-up one hour before use. Iron(III) ions can be analyzed at the part-per-million (ppm) level by forming a red-colored thiocyanate complex[9]. Standards ranging in concentration from 1 to 24 ppm were prepared using stock solutions of KSCN, 995 ppm iron(III) and distilled water. The absorbance of each of these solutions was obtained at the absorption maximum using each of the UV-Vis instruments studied. The wavelength of the absorbance maximum (479 nm) was obtained by scanning the absorbance of a ca. 4 ppm standard with the Cary 300 using the scanning feature to obtain a spectrum from 180-700 nm. The Vernier colorimeter utilizes four LED light sources and the LED used had a maximum wavelength of 470 nm which is reasonably close to 479 nm. A sample of each standard was placed in the same cuvette and analyzed in each of the spectrophotometers with the exception of the Red Tide which used a cuvette supplied with that spectrophotometer. Standards were analyzed in order from low to high concentration. A different operator was assigned to each spectrophotometer in order that all samples were ran by the same person on any given instrument. Blanks were run before the first sample and after the last sample. Two separate blanks were measured; one containing only distilled water, the other containing equal amounts of 1.5M KSCN and distilled water. Using the absorbance values collected for each concentration, a calibration curve was constructed for each instrument. Examples of the calibration curves are given in Figure 1.

The linear dynamic range is the linear portion of the calibration curve that can be used to obtain reproducible concentrations. The upper bound (limit of linearity or LOL) of the dynamic range is determined by inspecting the graph for the point where it deviates from linearity. For the Cary 300, the calibration curve was linear over the entire region accessible by the instrument (maximum absorbance = 4) and thus the LOL for the Cary 300 is 24 ppm. For the Genesys 20, the calibration curve is linear to 16 ppm.

Discussion

After the analyses were performed, figures of merit were determined for each instrument. The figures of merit calculated for each are shown below.

Instrument	Detection Limit (ppm)	Linear Dynamic Range (ppm)	Analytical Sensitivity	Calibration Sensitivity
Vernier	0.014	0.047-8	79	0.1764
GeneSys 20	0.0077	0.026-16	124	0.1744
Red Tide	0.0080	0.027-12	144	0.1677
Spectronic 200	0.19	0.63-12	13	0.1733
Colorimeter	0.05	0.15-10	90	0.1165
Cary 300	0.0015	0.0051-24	393	0.1738

Table 2- Figures of merit for the UV-Vis spectrometers in the Angelo State University Chemistry Department.

Detection limit	Linear Dynamic Range	Analytical Sensitivity
Cary 300	Cary 300	Cary 300
GeneSys 20	GeneSys 20	Red Tide
Red Tide	Red Tide	GeneSys 20
Vernier	Spectronic 200	Colorimeter
Colorimeter	Colorimeter	Vernier
Spectronic 200	Vernier	Spectronic 200

Table 3- Ranking of UV-Vis spectrophotometers based on key figures of merit

(ranked from best at top to poorest at the bottom).

As seen in Table 2, the Cary 300 spectrometer had the lowest detection limit, largest linear dynamic range, and largest analytical sensitivity which makes it excellent for quantitative work. This was expected because the Cary, which has photomultiplier tube detection and a double beam optical design, has the best features found on any of the six instruments

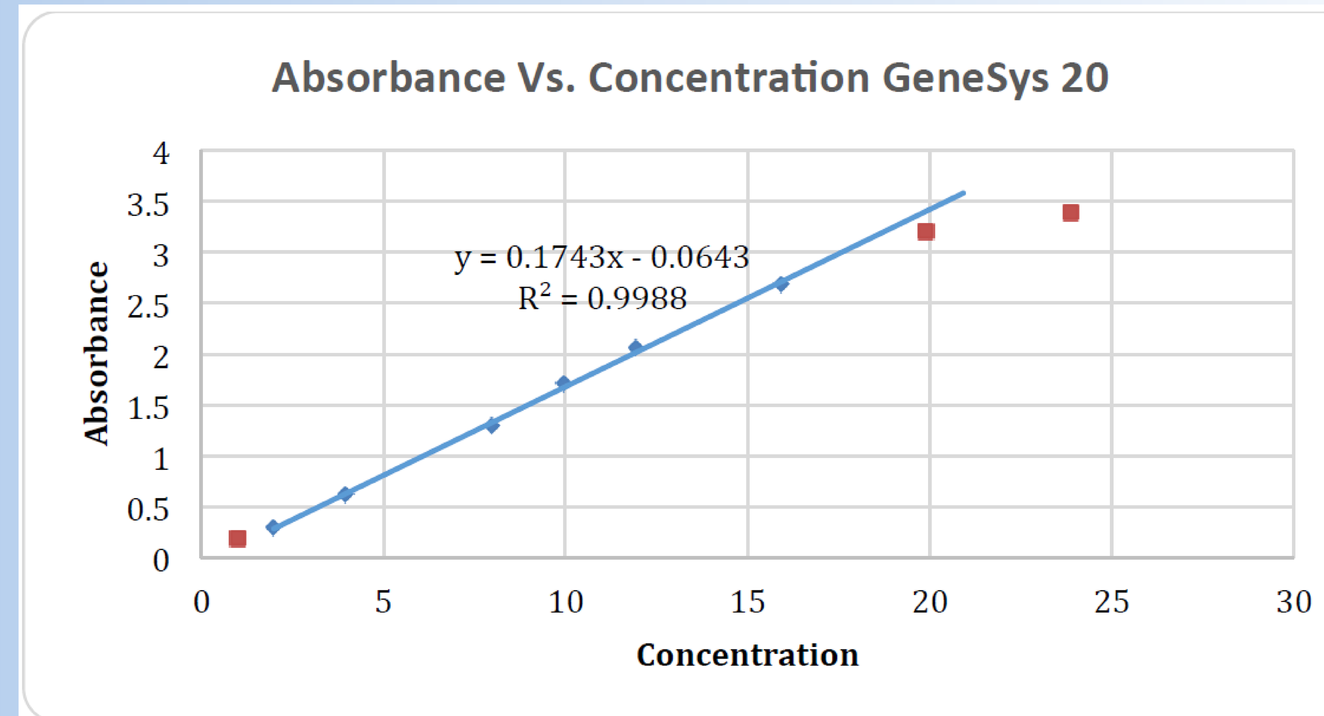
The GeneSys 20 produced a calibration curve with a large linear dynamic range and could be used over a wide range of concentrations with precision which makes it suitable for quantitative analysis as well. The re- sults were suitable for quantitative analysis at one-fifteenth the cost of the Cary 300. The remaining instru- ments produced results less suitable for quantitative analysis, but gave an insight into which instruments can be used for specific analyses. The colorimeter had limited wavelengths at which samples could absorb light and did not have a scanning spectrum feature. For a general chemistry lab in which the wavelength of absorption is already known, this spectrometer would produce valid results in teaching students how to create a calibration curve or a Beer's Law plot and be extremely cost-efficient for the department.

The Vernier spectrometer, the Spectronic 200, the Red Tide and the Cary 300 all have the ability to produce a spectrum that can be analyzed to determine the peak absorption wavelength and used for qualitative anal- ysis purposes. It is obvious that the Cary 300 is the best instrument to use for quantitative analysis of un- known samples as it has a large linear dynamic range and remains accurate over a large range of absorbance values. However it is also not plausible to purchase five or six of these instruments for class use because of the exceptionally high cost. To analyze samples when the peak absorption wavelength is unknown, the Vernier spectrometer and the Spectronic 200 are much less expensive and can still give reasonable data. The Spectronic 200 has a slightly lower LOQ than the Vernier but also costs four times as much.

Both instruments produce reliable data from around 0.5 to 2.0 absorbance units but then tail off. These in- struments provide another cost-efficient way of teaching students how to use a scanning spectrometer and to analyze unknown samples in a laboratory setting.

When looking strictly at the analytical and calibration sensitivities it is easy to determine the instruments that perform the best. The Cary 300 and GeneSys 20 both have the best analytical sensitivity and the same calibration sensitivity. The GeneSys 20 would be much more valuable if the instrument had a scanning fea- ture and could be used to determine the peak absorption wavelength. The other four instruments had lower analytical sensitivities and could be used for less demanding analysis of samples than the Cary 300 or the GeneSys 20. The calibration sensitivities were all very similar except for the colorimeter and Vernier spectrometer. A lowering of the calibration sensitivity is expected for the colorimeter as the excitation band that is absorbed is ca. 30 nm wide at one-half of maximum (FWHM) absorbance. All of the other spectro- photometers had a FWHM < 5nm.

Data



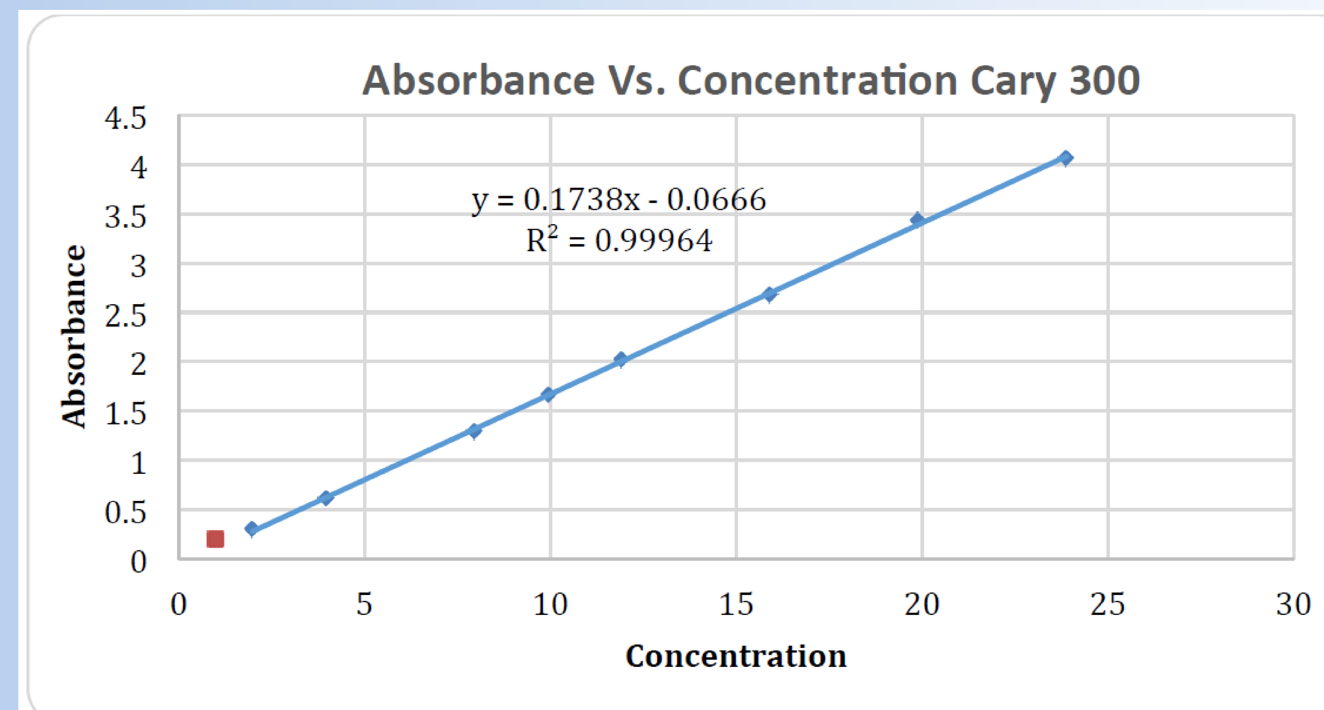


Figure 1- Typical Calibration Curves

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