

Statistical Analysis for Aqueous Cupric Sulfate Photometric Measurements

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Abstract

With respect to photometers, cuvettes and molarity, absorbance variability was determined for aqueous cupric sulfate. Molar absorptivity for 3x3 photometer/cuvette statistical design had confidence interval, $12.02 \leq \langle \alpha \rangle \leq 12.06 \text{ cm}^{-1} \text{ M}^{-1}$ at 95% level. With respect to variability in molar absorptivity, Two-way ANOVA revealed marginal photometer dependency, insignificant cuvette dependency, and insignificant photometer/cuvette interaction. Three-way ANOVA for absorbance variability revealed significant direct Beer's Law effect, insignificant direct effects for both photometer and cuvette, insignificant cuvette/molarity interaction, marginal molarity/photometer interaction, and significant cuvette/photometer interaction. Molarity measured for 0.0200 M unknown solution had percent error of 0.389, coefficient of variation(CV) of 0.468, and instrumental limit CV of 0.1.

Keywords: Photometer, cuvette, molarity, absorbance, cupric sulfate

1. Introduction

Photometric experiments provide interesting applications of Beer's Law in general chemistry and quantitative analysis courses. Others have used such methods in analysis of cupric[1], permanganate[2] and ferrous[3]ions. The usual experiment, (a)makes use a single combination of photometer and cuvette, (b) scans the absorbance versus wave length spectrum to locate appropriate absorbance maximum, (c) determines an absorbance versus concentration calibration curve for a standard solution set, and (d)yields the concentration of an unknown sample. By including three

spectrometers and three cuvettes, this study investigated variability inaccuracy and precision for mean molar absorptivity and determined the molarity of an unknown of cupric sulfate at 810 nm and 22⁰C. The absorbance versus concentration calibration curves were used to determine accuracy and precision of the concentration of a 0.0200M cupric unknown. Absorbance versus molarity calibration was performed using least-squares analysis of a five-fold standard solution set. Finally, two-way [4, 5] and three-way [4, 6] analysis of variance (ANOVA) were used to determine statistical significance for variability in absorbance.

2. Experimental

Three Thermo-Fisher Scientific spectrometers (Catalog # 4001) were allowed to warm up for 15 minutes. Wavelengths were selected and photometers were calibrated to 0% and 100% transmittance using de-ionized water. Absorbance measurements were obtained for standard 0.100 M aqueous cupric sulfate using three-fold replications with a single cuvette. These data are reported in Table 1 for wavelengths 750 – 840 nm.

Table 1. Absorbance versus wavelength(nm) for 0.10 M cupric sulfate

Wavelength (nm)	Absorbance		
	A	B	C
750	1.044	1.041	1.042
760	1.097	1.096	1.099
770	1.141	1.141	1.141
780	1.173	1.175	1.172
790	1.196	1.197	1.195
800	1.209	1.209	1.206
810(Λ_{MAX})	1.215	1.212	1.212
820	1.210	1.210	1.209
830	1.198	1.199	1.195
840	1.181	1.186	1.179
850	1.160	1.164	1.156

Thermo-fisher Scientific Catalog #4001: Spectrometers A(Serial # 3SGQ355002), B(Serial # 3SGT202007), C(Serial # 3SGT202006), cuvette #1, and room temperature 22⁰C.

2.1 Preparation of standard cupric sulfate solutions

The cupric sulfate solutions were prepared as follows. Technical grade cupric sulfate hexa-hydrate (molar mass 249.685 g) was obtained from Fisher Chemicals. With analytical balance 24.9691 g were weighed and dissolved in water to make 0.1000 M solution using volumetric flask. Then five additional solutions of molarities- 0.050 M, 0.026 M, 0.020 M, 0.012 M and 0.006 M– were prepared using quantitative dilutions with volumetric pipets and volumetric flasks. The 0.020 M solution served as an unknown cupric sulfate and the other five solutions provided a standard solution set.

2.2 Maximum absorbance wavelength

The wavelength of maximum absorbance Λ_{MAX} occurred at 810 nm as determined from plots of absorbance against wavelength. Measured absorbance means given in Tables 2 and 3 were collected at this wavelength using three-fold replications.

Table 2. Mean absorbance versus molarity for standard solutions, cuvettes and photometers^A

Molarity	Absorbance		
	A1	B1	C1
0.006	0.0707	0.0697	0.0700
0.012	0.1470	0.1433	0.1433
0.026	0.3177	0.3127	0.3100
0.050	0.6007	0.5993	0.5970
0.100	1.2063	1.2007	1.1987
A2	B2	C2	
0.006	0.0693	0.0687	0.0707
0.012	0.1407	0.1407	0.1447
0.026	0.3153	0.3123	0.3120
0.050	0.6060	0.6023	0.6047
0.100	1.2033	1.2013	1.2007
A3	B3	C3	
0.006	0.0653	0.0710	0.0703
0.012	0.1390	0.1487	0.1447
0.026	0.3123	0.3137	0.3150
0.050	0.6030	0.6007	0.6007
0.100	1.1980	1.2033	1.2056

^A Mean absorbance using 3-fold replications versus molarity, cuvettes and spectrometers.

3. Calculations

The calculations required linear least-squares analysis of absorbance versus cupric sulfate concentration for 3X3 combinations of cuvette and spectrometer. Both One-way and Two-way ANOVA[4,5] were used to evaluate significance of variation in molar absorptivity with respect to photometers and cuvettes. Two-way ANOVA was used to assess variability in mean absorbance, and was also used to examine variability in accuracy and precision for the molarity of 0.0200 M cupric sulfate solution. Three-way ANOVA[4, 6] was used to evaluate significance of absorbance variability with respect to photometers, cuvettes and solution concentrations.

3.1 Least-squares calibration curves

In the least-squares analysis absorbance (Y) was the dependent variable and concentration was the independent variable (X). The standard solution set had five different molarities. Slope **b** and intercept **a**, regression coefficient **S_R**, standard deviations **S_A** for intercept and **S_B** for slope, and

correlation coefficients were determined for each of the 3x3 cuvette/photometer combinations. The mean absorbance $\langle \text{Abs} \rangle$ and its standard deviation S_{Abs} for the unknown cupric sulfate solution are reported in Table 3. The cupric sulfate mean molarity C and standard deviation S_C given in Table 5 for each cuvette/photometer combination were determined from measured absorbance and $(A - a)/b$ for each unknown solution. The measure of accuracy, percent error, $\% E_X = 100 (\langle X \rangle - X_{\text{True}}) / X_{\text{True}}$, and the measure of precision, coefficient of variation, $CV = 100 S_X / \langle X \rangle$ were used to calculate the $\%E$ and CV given in Table 5. None of these determined molarities were outliers. Deviations from 1.0 in calculated least-squares correlation coefficients were less than 0.1 ppt. This level of linearity supported using the slope b to calculate molar absorptivity reported in Table 4. None of these mean molar absorptivity values were outliers.

Table 3. Mean absorbance for unknown solution ^A

	Absorbance(810 nm)		
	A1	B1	C1
$\langle \text{Abs} \rangle$	0.2427	0.2407	0.2393
S_{Abs}	5.774×10^{-4}	5.774×10^{-4}	5.774×10^{-4}
	A2	B2	C2
$\langle \text{Abs} \rangle$	0.2430	0.2397	0.2410
S_{Abs}	4.359×10^{-3}	4.774×10^{-4}	2.000×10^{-3}
	A3	B3	C3
$\langle \text{Abs} \rangle$	0.2363	0.2420	0.2423
S_{Abs}	5.774×10^{-4}	1.000×10^{-3}	5.774×10^{-4}

^AAbsorbance means with three-fold replication for 0.0200 M $\text{CuSO}_4(\text{aq})$, cuvettes and spectrometers.

Table 4. Beer's Law molar absorptivity for standard solutions ^A

	A1	B1	C1
α	12.06	12.03	12.00
S_α	3.668×10^{-2}	3.089×10^{-2}	1.305×10^{-2}
	A2	B2	C2
α	12.07	12.05	12.02
S_α	4.738×10^{-2}	2.635×10^{-2}	3.182×10^{-2}
	A3	B3	C3
α	12.04	12.01	12.06
S_α	5.329×10^{-2}	3.221×10^{-2}	2.335×10^{-2}

^ABeers Law molar absorptivity α ($\text{M}^{-1} \text{cm}^{-1}$) ^{A,B} and standard deviation S_α for standard solutions set using cuvettes, and photometers with 3-fold replication for each absorbance measurement.

^B Least-squares analysis for standard solution set using $Y = a + b X$, as Beer's Law absorbance equation.

Table 5. Unknown cupric molarity X_C , standard deviation S_C , percent error% E_C , and percent coefficient of variation CV_C for spectrometers and cuvettes Spectrometer and cuvette

	A1	A2	A3
X_C	0.0200	0.0202	0.0200
S_C	2.607×10^{-4}	3.363×10^{-4}	3.792×10^{-4}
%E	-0.104	1.135	-0.300
CV	1.305	1.661	1.902
	B1	B2	B3
X_C	0.0202	0.0201	0.0200
S_C	2.199×10^{-4}	1.874×10^{-4}	2.298×10^{-4}
%E	1.205	0.498	0.175
CV	1.088	0.933	1.147
C1	C2	C3	
X_C	0.0201	0.0200	0.0202
S_C	0.932×10^{-4}	2.269×10^{-4}	1.659×10^{-4}
%E	0.450	0.175	0.730
CV	0.464	1.132	0.824

Table 6. Two-way ANOVA for mean absorbance of unknown^A Main factors F_{Exp} F_{Crit} (5 % level Significant

Photometer 0.81943.55 No
Cuvette 0.03043.55 No

Factor interaction

Photo-cuvette 8.389 2.66 Yes

^AThe unknown is 0.020 M cupric sulfate; S = standard deviation; Row factor R (Photometers A, B and C); and Column factor C (Cuvettes 1, 2 and 3).

Table 7. Two-way ANOVA for variability in mean molar absorptivity of cupric sulfate with respect to spectrometers and cuvettes.

Main factors	F_{exp}	F_{Crit} (5 % level)	Significant
Spectrometer	3.40	3.27	Yes(marginal)
Cuvette	0.81	3.27	No
Factor interaction	F_{exp}	F_{Crit} (5 % level)	Significant
Spect-cuv	2.50	2.64	No

Table 8. Two-way ANOVA for variability in molarity of 0.02 M cupric sulfate with respect to spectrometers and cuvettes

Main factors	F_{exp}	$F_{Crit(5\% \text{ level})}$	Significant
Spectrometer	0.073	3.55	No
Cuvette	0.31	3.55	No
Factor interaction	F_{exp}	$F_{Crit(5\% \text{ level})}$	Significant
Spect-cuv	0.73	2.96	No

Table 9. Three-way ANOVA for variability in mean absorbance for spectrometer, sample cuvette and cupric sulfate concentration

Main factors	F_{exp}	$F_{Crit(5\% \text{ level})}$	Significant
Spectrometer	1.29	3.63	No
Cuvette	0.023	3.63	No
Concentration	7.2×10^5	3.01	Yes
Factor interactions	F_{exp}	$F_{Crit(5\% \text{ level})}$	Significant
Spect-cuv	14.03	3.01	Yes
Spect-conc	2.9	2.59	Yes(marginal)
Cuv-conc	1.6	3.59	No

3.2 One-way ANOVA

Three separate One-way ANOVA calculation [4] were used, one for mean absorbance of the unknown cupric sulfate solution reported in Table 3, another for mean molar absorptivity $\langle \alpha \rangle$ given in Table 4, and a third for the mean molarities given in Table 5 for the unknown solution. There were nine individual means $\langle X \rangle$ and standard deviations S_X for each of these three problems. This was called a One-way nine levels ANOVA problem. The ANOVA method provided a systematic method to answer the following question. Were variations among the mean dependent variable sufficiently large to indicate that different samples were tested? Our statistical Null Hypothesis stated that all these samples were subjected to the same treatment and that all variations among the means were both small and randomly distributed. A Fisher F-test [9] was used to decide whether to accept or reject this Null Hypothesis. The Fisher quotient ($F_{exp} = S_B^2 / S_W^2$) was calculated and compared to the critical quotient F_{crit} which came from the Fisher Statistics Table [9] and depended only upon the number of degrees of freedom DF_B and DF_W , and the desired probability significance level. If F_{exp} were less than or equal to F_{crit} the Null Hypothesis was accepted; otherwise the Alternative Hypothesis was accepted. S_B^2 was the between groups variance and S_W^2 was the within groups variance. The Central Limits Theorem of statistics insures that if variances S_B^2 and S_W^2 were both valid statistical estimates of the same normal population variance σ^2 , the F_{test} would give S_B^2/S_W^2 equal to ≈ 1.0 and the Null Hypothesis should be accepted.

3.3 Two-way ANOVA

Two-Way ANOVA[4, 5] was very similar to One-Way ANOVA. The calculations determined variability introduced into a measured dependent variable through use of different cuvettes and photometers. Three dependent variable cases were separately considered. First, mean absorbance for the unknown cupric sulfate reported in Table 3, next mean molar absorptivity for standard solutions given in Table 4, and finally mean molarity for unknown copper sulfate given in Table 5. The main independent variables, called row(**a**) or column(**b**) factors, were cuvettes (1, 2 and 3) and photometers (A, B and C). This problem was called a two factor 3x3 ANOVA with interaction. For the two main factors, **a** and **b**, Fisher F quotient F_a and F_b had to be calculated. And for each pair of main variables, **a** and **b**, there was one between-interaction F quotient, F_{ab} that had to be calculated. These experimental F quotients were compared to their critical F values from Fisher Statistics Tables[9] at 5% probability level according to their numerator DF_{NUM} and denominator DF_{DENOM} degrees of freedom. Statistical Null Hypothesis and Alternative Hypotheses were defined for each calculated F quotient. Each Null Hypothesis NH was accepted if its F_{exp} was less than or equal to its F_{crit} . But the Alternative Hypothesis AH was accepted if F_{exp} was greater than its F_{crit} .

3.4 Three-way ANOVA

Three-way ANOVA[4, 6] with two-way interaction was similar to Two-Way ANOVA. Independent variables, called factors, **a**, **b**, and **c**, were cuvettes (1, 2 and 3), photometers (A, B and C), and solution molarities (1, 2, 3, 4 and 5). This type of ANOVA, called a 3x3x5 ANOVA with two-way interactions, contained three **a** levels, three **b** levels and five **c** levels. Using absorbance data given in Table 2 calculations were made. Now three single factor F quotients F_a , F_b and F_c along with three between-factor interaction F quotients, F_{ab} , F_{ac} and F_{bc} had to be calculated. These calculated F quotients were compared to critical F values from Fisher Statistics Tables[9] at 5% probability level according to the numerator DF_{NUM} and denominator DF_{DENOM} degrees of freedom. Each Null Hypothesis NH was accepted if its F_{exp} was less than or equal to F_{crit} , but the Alternative Hypothesis AH was accepted if its F_{exp} was greater than F_{crit} .

4. Discussion

The following items are discussed, (a) determination of instrumental precision limits for the standard and unknown cupric sulfate solutions, (b) determination of the mean molar absorptivity for cupric sulfate at 810 nm, (c) and analysis of absorbance variability versus concentration, photometers and cuvettes.

4.1 Instrumental precision limits

Instrumental precision limits[10] for the standard 0.1000 M and unknown (0.0200 M) cupric sulfate solutions were calculated. The law of variance equation for molarity M was written in terms of the instrument tolerances. S_W was 0.1 mg for analytical balance and S_V was 0.3 mL for a one-liter volumetric flask. The cupric sulfate hexa-hydrate mass was 24.968 g for one liter 0.1000 M solution: $S_M = M[(S_W/W)^2 + (S_V/V)^2]^{1/2}$. The S_M was 3.0×10^{-5} moles/L and its coefficient of variation, CV was 0.030. The dilution equation, $M_C V_C = M_D V_D$, was used to calculate precision of 1:5 dilution of the standard 0.1000 M solution. For a 0.0200 M cupric sulfate S_M was 2.2×10^{-5} M and its coefficient of variation CV_{instr} was 0.11.

4.2 Variability in absorbance of unknown cupric sulfate

Mean absorbance variability in the cupric sulfate unknown was first examined using outlier tests and followed with Two-Way ANOVA. The nine mean absorbance values reported in Table 3 for cupric sulfate were examined for outliers. Dixon's method revealed no outliers, but Grubb's method flagged the absorbance value, 0.2363 as an outlier. Two-Way ANOVA was applied to absorbance given in Table 3 to explore effects of cuvette and photometer upon absorbance variability. The photometer's effect upon absorbance variability was insignificant because its measured Fisher F quotient, 0.8194 was less than 3.55, the critical F at a 5% confidence level. The cuvette's effect upon absorbance variability was also insignificant because its measured Fisher F quotient, 0.0304 was less than 3.55, the 5% confidence level F_{Crit} value. However, the photometer/cuvette's interaction effect upon absorbance variability was significant because its measured Fisher F quotient, 8.3866 was greater than 2.66, the 5% confidence level F_{Crit} value.

4.3 Concentration of the unknown cupric sulfate

Accuracy and precision in concentration of the unknown cupric sulfate solution are discussed. Absorbance variability was impacted by significant cuvette-photometer interaction, but the least-squares calibration procedure successfully minimized the influence of this cuvette/photometer interaction upon the determined unknown molarities. Two-way ANOVA was applied to the molarity reported in Table 5 for the unknown cupric sulfate solution. Two main factors, photometers (A, B and C) and cuvettes (1, 2 and 3), and the photometer/cuvette interactions were considered; ANOVA results are presented in Table 8. The photometer's effect upon variability in mean molarity of the unknown solution was statistically insignificant at a 5% confidence level because its Fisher[9] F quotient, 0.073 was less than the critical F_{Crit} value of 3.55. The cuvette's effect upon variability in the mean molarity of the unknown solution was statistically insignificant at a 5% confidence level because its Fisher F quotient, 0.31 was smaller than 3.55, the F_{Crit} . The effect of photometer/cuvette interaction upon variability in the mean concentration of the unknown was statistically insignificant at a 5% confidence level because the Fisher F quotient, 0.73 was less than 2.96, the F_{Crit} . Furthermore, none of the molarities reported in Table 5 for unknown solution were outliers. The mean cupric sulfate molarity and standard deviation were 2.009×10^{-2} M and 1.027×10^{-4} M, respectively for the unknown. Accuracy as percent error was 0.439 and precision CV was 0.51 for the cupric sulfate unknown. The instrumental limit precision standard deviation and CV, respectively were 2.2×10^{-5} M and 0.11 %.

4.4 Molar absorptivity of cupric sulfate

For a one cm length cuvette cell molar absorptivity at 810 nm is the slope of absorbance plotted against molarity. The standard solution set consisted of five concentrations ranging from 0.006M to 0.100 M. With use of three cuvettes and three spectrometers nine least-squares calibration plots were obtained from the absorbance versus concentrations given in Table 2. The measured Beer's law mean molar absorptivity $\langle \alpha \rangle$ coefficients and standard deviations S_{α} are presented in Table 4. None of these mean α coefficients which ranged from 12.00 to 12.06 $\text{cm}^{-1} \text{M}^{-1}$ were outliers. Their mean, 12.04 $\text{cm}^{-1} \text{M}^{-1}$, median 12.04 $\text{cm}^{-1} \text{M}^{-1}$, and standard deviation $2.45 \times 10^{-2} \text{cm}^{-1} \text{M}^{-1}$ belong to a normal distribution having Pearson coefficient, -0.273. The coefficient of variation CV was 0.20 and the 95% level statistical confidence interval was, $12.01 \leq \langle \alpha \rangle \leq 12.06 \text{ cm}^{-1} \text{M}^{-1}$. One-way ANOVA of mean molar absorptivity was performed. Results were as follows: $N = 9$, $S_W = 3.387 \times 10^{-2}$, $S_B = 4.223 \times 10^{-2}$, $DF_{\text{Num}} = 8$, $DF_{\text{Denom}} = 18$, $F_{\text{exp}} = 1.554$, and 5% level $F_{\text{Crit}} = 2.51$. Observed variations among the nine

mean molar absorptivity $\langle \alpha \rangle$ were statistically insignificant at a 5% confidence level. Two-way ANOVA results for mean molar absorptivity are given in Table 7. The photometer's F quotient, 3.40 was larger than, 3.27 the critical F. This amount of variability in molar absorptivity was marginally statistically significant. The cuvette's F quotient, 0.81 was smaller than, 3.27 the critical F, so variability in molar absorptivity was statistically insignificant. The photometer/cuvette's interaction F quotient, 2.50 was smaller than, 2.64 the critical F, so photometer/cuvette's interaction produced insignificant variability in molar absorptivity. These statistical descriptions of variability for measured molar absorptivity were consistent with absence of outliers among the measured molar absorptivity and least-square correlation coefficients each being close to 1.0.

4.5 Absorbance versus concentrations, spectrometers and cuvettes

Three-way ANOVA provided another way to monitor consistency in the measured absorbance given in Table 2 relative to variations in concentration, spectrometers and cuvettes. Beer's law requirement of linear dependence of absorbance upon concentration was confirmed, and none of the measured molar absorptivity coefficients were outliers. Three-way ANOVA statistical analysis results are given in Table 9.

4.5.1 Direct effects

The photometer's F factor, 1.29 was smaller than the critical F, 3.63 so the photometer's effect upon absorbance variability was statistically insignificant. The cuvette's F factor, 0.023 was smaller than its critical F 3.63, so cuvette's effect upon absorbance variability was statistically insignificant. However, the concentration F factor, 7.2×10^5 was much greater than its critical F, 3.01 so there was a strong direct Beer's Law concentration effect on absorbance variability.

4.5.2 Interaction effects

Statistical significance of interaction effects were measured. The cuvette/photometer's interaction F factor, 14.0 was greater than the critical F, 3.01 so there was a significant cuvette/spectrometer interaction effect upon absorbance variability. The spectrometer/concentration interaction F factor, 2.9 was only marginally greater than the critical F, 2.59 so the photometer/molarity interaction effects upon absorbance variability were marginally statistically significant. Cuvette/molarity interaction F factor, 1.6 was less than the critical F, 3.59 so cuvette/molarity interaction effect upon absorbance variability was statistically insignificant.

5. Summary

This study used least-squares analysis to calculate absorbance versus molarity calibration curves and molar absorptivity for 3X3 photometer/cuvette combinations. Absorbance variability was determined at 22°C for a aqueous cupric sulfate with respect to photometers, cuvettes and solute concentration.

5.1 One-way ANOVA showed that variability in the mean molar absorptivity was statistically insignificant (5% level F Tests), and Two-way ANOVA confirmed that variability in mean molar absorptivity with respect to cuvette and to cuvette/photometer interaction were statistically insignificant. A marginal level of variability in the mean molar absorptivity was found for the photometer.

5.2 Three-way ANOVA extended to include variability in absorbance with respect to concentration provided significant support for the Alternative Statistical Hypothesis known as Beer's Law. With

respect to both cuvettes and photometers absorbance variabilities were statistically insignificant, but interaction effects were significant for both photometer/cuvette and photometer/concentration but not for cuvette/ concentration.

5.3 Two-Way ANOVA revealed absence of significant variability in the determined unknown molarity with respect to cuvette, photometer, and cuvette/photometer interaction. Consequently, the conventional absorbance/standard solution calibration procedure provided accurate and precise determination of the molarity of the unknown.

5.4 Absorbance measured for 0.020 M unknown cupric sulfate solution yielded concentration accuracy with percent error 0.389, CV precision coefficient 0.468, and a corresponding CV instrumental limit 0.11.

5.5 Finally, measured molar absorptivity coefficients α ranged from 12.00 to 12.06 $\text{cm}^{-1} \text{M}^{-1}$, with mean 12.04 $\text{cm}^{-1} \text{M}^{-1}$, standard deviation $2.353 \times 10^{-2} \text{cm}^{-1} \text{M}^{-1}$, coefficient of variation 0.20, and a 95% level statistical confidence interval, $12.01 \leq \alpha \leq 12.06 \text{cm}^{-1} \text{M}^{-1}$.

6. References

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