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Experiments
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CONSTRUCTION OF JOB'S PLOT IN CASE OF HIGH SPECTRAL OVERLAP BETWEEN THE REACTANTS AND THE COMPLEX: APPLICATION OF MULTIWAVELENGTH LINEAR REGRESSION ANALYSIS USING THE CU-EDTA SYSTEM

[1]Agha Arslan Wasim, [1]Muhammad Nasiruddin Khan,
[2]Muhammad Farooq Wahab, [1]Saba Fazal-ur-Rehman
[1]University of Karachi (Pakistan)
University of Texas Arlington (USA)

Abstract. Job's method is extensively used to determine the stoichiometry of complex ions both in the field of education and research. However, the shape of the Job's plot generally does not match to what is shown in the textbooks due to simultaneous absorption by other species involved in the equilibria. The conventional correction method deals the problem by assuming that no reaction would have occurred among the reactants and thus uses their formal concentrations for the correction of empirical data. The assumption is contradictory as, in solution equilibria, formal concentrations differ substantially from the respective equilibrium concentrations. Alternatively, multiwavelength linear regression analysis is used to determine the equilibrium concentrations of reactants in the system. The two correction methods, when applied on Cu-EDTA system, showed a significant difference between them. The comparative study is designed to evaluate the correction methods. Moreover, Microsoft Excel® was incorporated to transform tedious correction into quick and interesting processing of data.

Keywords: job plot; continuous variation; absorbance correction; stoichiometry

Introduction

The importance of complexation equilibria in chemical education and research cannot be overemphasized. The experimental determination of complex ion stoichiometry by UV-Vis spectrophotometry is a popular experiment in chemistry throughout the world. The method of continuous variation, the Job's method, has been extensively used in the determination of complex ions stoichiometry. A diverse range of systems have been studied by using this method. Most common systems are the inorganic complexes (Carmody, 1964; Hill & MacCarthy, 1986) dyes-surfactant associates (Karukstis et al., 1998), and the protein-protein complexes (Ingham, 1975). Besides determining stoichiometry, this method has also been used to study the mechanism of chelation process (Huang et al., 2003). Most of the experiments related to Job's plot are used to determine the stoichiometry of the complex ions. For the study of a general complexation reaction between a metal (M) and a ligand (L)

$$mM + nL \rightleftharpoons M_mL_n$$

A series of solutions is prepared using equimolar solution of M and L such that the sum of formal concentrations of M and L is kept constant whereas the L to M ratio is varied systematically within the series. In principle, any physical property which is directly related to complex ion concentration can be used to construct the Job's plot (Gil & Oliveria, 1990). The most commonly used technique is UV-Vis spectrophotometry of the complex ion using the Beer's law. The plot of absorbance against the volume fraction of M or L results in a Job's plot. It may either possess maxima or minima at a volume fraction which is used to determine the stoichiometry of complex ion.

A problem arises in the construction of the Job's plot when reactants absorbs at the selected wavelength which is normally the wavelength for complex. The nice inverted V shape is disturbed which might also affect the obtained results. There are methods reported for the correction of such spectral interference, but they deal the problem using formal concentrations of the reactants to estimate their absorbance contribution and correction. This contradicts the concept of solution equilibria. A better approach would be to use the equilibrium concentrations of species for the absorbance corrections. In this study Cu-EDTA complexation equilibria was selected to illustrate the concept of absorbance correction using equilibrium concentrations rather than their formal concentrations. This system showed significant spectral overlap, the unbound copper ion (Cu^{2+}) absorbs at essentially all the wavelengths that can be used to study the complex, i.e., there is a significant spectral overlap between Cu^{2+} and Cu-EDTA as shown in Fig. 1. The unbound ethylenediaminetetraacetic acid does not absorb light at the wavelengths selected for the analysis. The difference between the conventional and the proposed method of absorbance correction was elaborated when both methods were processed on the experimental data obtained for this system. The comparison revealed that there is a significant difference between the two correction methods. Moreover, the propagation of error to the conditional formation constant emphasized the use of equilibrium concentrations for the correction of measured absorbance.

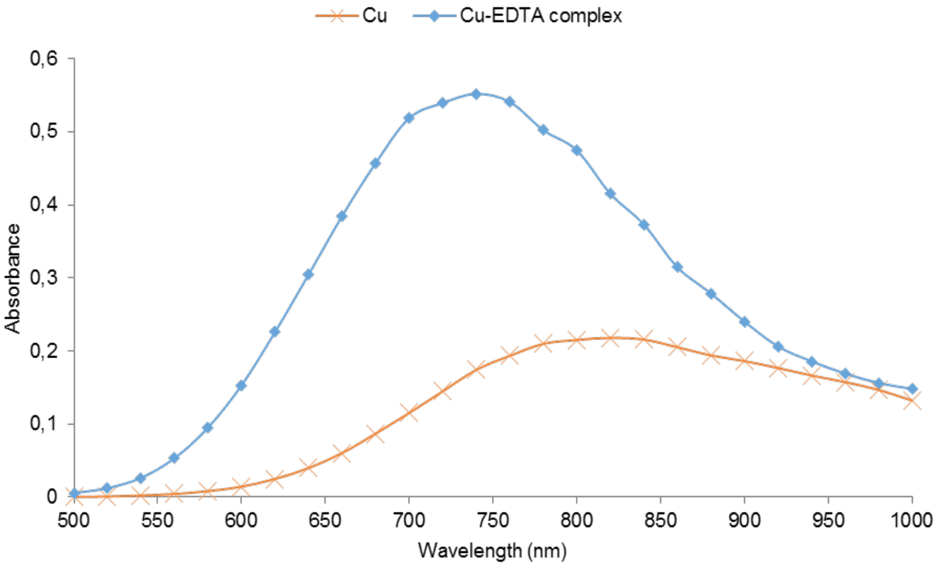


Figure 1. Visible spectra of Cu^{2+} ion (0.02M) and Cu-EDTA Complex (0.01 M) showing significant spectral overlap for the two species

Materials and methods

Equipment

Shimadzu 1601 UV-Vis spectrophotometer was used to measure the absorbance values of the series of solution in glass cells, 1cm pathlength. The data acquisition was carried out using the software UVPC39 installed in the computer interfaced with the spectrophotometer. The data was copied to MS excel spread sheet for further calculations. pH of the solution was measured using Hanna instruments pH 211 equipped with a combined glass electrode.

Chemicals

All chemicals used in this experiment were general laboratory grade reagents, mostly available in an undergraduate laboratory. Copper (II) sulfate-pentahydrate (Avonchem), disodium salt of ethylenediaminetetra-acetic acid (Na_2EDTA. Dihydrate) (Avonchem), sodium Acetate (BDH), Acetic acid (Avonchem), HCl (Fluka) and KCl (BDH) and distilled water were used to prepare the series.

Experimental procedure

0.1 M stock solutions of Cu^{2+} and Na_2EDTA were prepared using the respective salts and distilled water, no acid or base was added at the stage of stock preparation. The stock solutions were standardized using reported methods

(Christian, 2004; Mendham, 2000). A series of eleven solutions was prepared in which Cu to EDTA stock volume ratio (V_{Cu}: V_{EDTA}) varied from 0:10 to 10:0. The sum of the stock volumes was fixed to 10.0 mL and the final volume of each solution was made up to 50.0 ml in a volumetric flask using pH 4.7 buffer. The standard solution of Cu-EDTA complex (0.01 M) was prepared by using the stock solutions of Cu^{2+} and EDTA. The concentration of Cu^{2+} ions was 0.01 M and concentration of EDTA was nine times the Cu^{2+} concentration, whereas, the pH was maintained at 4.7 using respective buffer solution. pH 4.7 buffer was prepared by dissolving equal volumes of 0.2 M acetic acid and 0.2 M sodium acetate solution and the pH was finally adjusted to the labeled value by using the HCl or sodium hydroxide solution. Each solution was scanned in a UV/Vis spectrophotometer from 500 and 1000 nm. The wavelength interval for scanning was 5nm and the respective buffer was used as blank.

Result and discussion

It is generally considered that the unbound reactants present in the series do not absorb at the selected wavelength. Unfortunately, the nice Λ shape is generally not seen for the Job's plot when there are multiple absorbing species involved in the equilibria. In case of spectral overlap of reactants and the complex, the measured absorbance would be the accumulated absorbance of all species present in the system. Such a plot can still be used for determining the stoichiometry; however, the students can be left wondering as to why their plot does not match the *idealized* shapes in the textbook. In this case concept of absorbance correction by a blank becomes ambiguous because we do not know the exact concentration of absorbing species besides the complex. Thus, the absorbance of unbound reactants must be dealt in another way. We observed same behavior when we incorporated Job's plot experiment using copper EDTA in our analytical chemistry laboratory course. This led us to think about the concept of correcting absorbance in case of severe spectral overlap of components in the mixture.

The general texts discussing this method use the following equation for the correction of absorbance (Harris, 1999; Hill & MacCarthy, 1986; Hogfeldt, 1979):

$$A_{corr} = A_{meas} - (\epsilon_M C_M + \epsilon_L C_L)b \quad (1)$$

where, A_{corr} is the corrected absorbance used to construct the jobs plot, A_{meas} is the measured absorbance. The respective molar absorptivity of metal and ligand, at the λ_{max} of complex are represented by ϵ_M and ϵ_L, respectively. The pathlength of the cell is b, whereas, C_M and C_L are the *formal* concentrations of the metal and ligand in a solution of the series respectively. The correction method assumes that no reaction would have occurred between the reactants

(Gil & Oliveria, 1990; Hill & MacCarthy, 1986); therefore, the formal concentrations of reactants can be used in Eq. (1). This assumption is highly oversimplified. It ignores the formation of complex which can result in a significant difference between the equilibrium concentrations of the unbound reactants and their respective formal concentrations. Therefore, one can easily interpret that this assumption would result in underestimation of the corrected absorbance for complex ion. Moreover, the use of such corrected absorbance to evaluate conditional formation constant will lead to the propagation of error in the resulting values. We have designed an experiment that helps the students to understand the concepts of simultaneous analysis, multiwavelength linear regression and a relatively better way to correct background absorbance where simple blank corrections are inadequate.

Instead of using formal concentrations, this study suggests a correction method which relies on determining the equilibrium concentrations of unbound reactants which are then used in Eq. (1) to determine the corrected absorbance. The equilibrium concentrations are determined using Multiwavelength Linear Regression Analysis, a reported method, for the simultaneous analysis of a mixture using spectrophotometry (Blanco et al., 1989). This method is based on Eq. (2):

$$\frac{A_{mix}}{A_{M\ std}} = \frac{C_1}{C_{M\ std}} + \left(\frac{A_{ML\ std}}{A_{M\ std}}\right)\frac{C_2}{C_{ML\ std}} \tag{2}$$

where, $A_{mix}A_{mix}$ is the absorbance of the solution containing a mixture of complex ion and the unbound reactant. $A_{M\ std}$ and $C_{M\ std}$ are the absorbance and concentration of standard solution of the interfering reactant, respectively. $A_{ML\ std}$ and $C_{ML\ std}$ are the respective absorbance and concentration of standard solution of complex ion. C_1 and C_2 are the equilibrium concentrations of unbound interfering reactant and complex ion in the mixture respectively.

The absorbance of each solution of a series prepared to construct Job's method is scanned for a range of wavelengths. The standard solution of complex ion and unbound reactant are also scanned for the same wavelengths. The resulting data is then used to construct $\frac{A_{mix}}{A_{M\ std}}$ vs. $\frac{A_{ML\ std}}{A_{M\ std}}$ plot which should be a linear curve for each solution.

Table 1. Selected data of Absorbance ratio for the series and standard solutions. Volume fraction of Cu^{2+} increases from 0 to 1 from solution 1 to XI respectively

λ (nm)	$\left(\frac{A_{ML\ std}}{A_{M\ std}}\right)^{1*}$	Absorbance Ratio $\left(\frac{A_{mix}}{A_{M\ std}}\right)^{2\dagger}$										
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI
1000	1.1218	0.0045	0.2330	0.4523	0.6679	0.9259	1.1657	1.2481	1.2564	1.1808	1.1271	1.0000
970	1.0690	0.0079	0.2216	0.4175	0.6325	0.8534	1.0651	1.1604	1.1381	1.0953	1.0487	1.0000
870	1.4927	0.0050	0.3290	0.6136	0.8861	1.1360	1.3511	1.4136	1.3486	1.2428	1.1501	1.0000
770	2.6735	0.0050	0.6330	1.1510	1.5920	1.9245	2.2370	2.2450	1.9810	1.7150	1.4065	1.0000
730	3.5313	0.0063	0.8459	1.5117	2.0752	2.5502	2.8661	2.8294	2.4713	2.0373	1.6014	1.0000
670	5.8642	0.0137	1.4266	2.5391	3.5309	4.2195	4.8395	4.6859	3.8848	3.0604	2.1317	1.0000
570	11.9016	0.1803	3.0000	5.1803	7.3115	8.6721	9.6066	9.5574	7.7705	5.9508	3.8033	1.0000

* Absorbance ratios for Cu-EDTA complex and Cu^{2+} standard solutions at various wavelengths
** Absorbance ratios of a mixture and Cu^{2+} standard solution at various wavelengths

The absorbance ratio plots for the series are shown in Fig. 2. The slope and intercept of each curve were determined which were then used to determine the concentrations C_1 and C_2 for each solution. The correlation coefficients for the dependent $\left(\frac{A_{mix}}{A_{M\ std}}\right)$ and independent $\left(\frac{A_{ML\ std}}{A_{M\ std}}\right)$ variables were determined using CORREL formula. The values were ranging from 0.9989 to 0.9997. The best values of correlation coefficients were found when the data from 560 – 1000 nm was used for the calculations. Most of the calculations were performed using Microsoft excel. The slope and intercept from the absorbance ratio data was calculated using LINEST formula and the graphs were also plotted using the spreadsheets as shown in Fig. 4.

The molar absorptivity of Cu^{2+} ($\epsilon_{Cu^{2+}}$) was determined using the concentration and absorbance of the standard solution for Cu^{2+} ions at the λ_{max} of complex (730 nm). Table 1 summarizes the experimental data. The absorbance contribution by the unbound Cu^{2+} ion was corrected using Eq. (3); the modified form of Eq. (1).

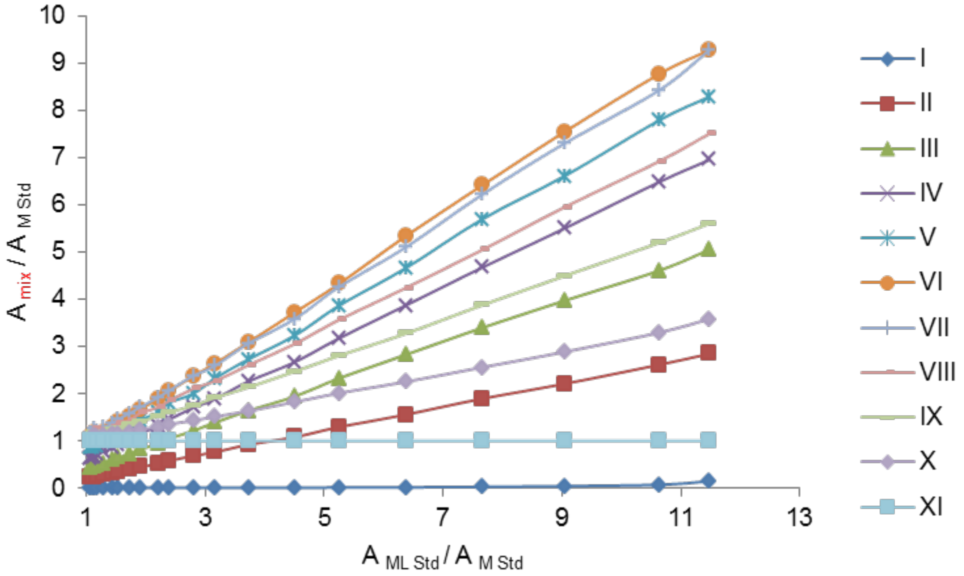


Figure 2. Plots of absorbance ratio for the series. From I to XI, V_{Cu}: V_{EDTA} changes from 0:10to 10:0 respectively

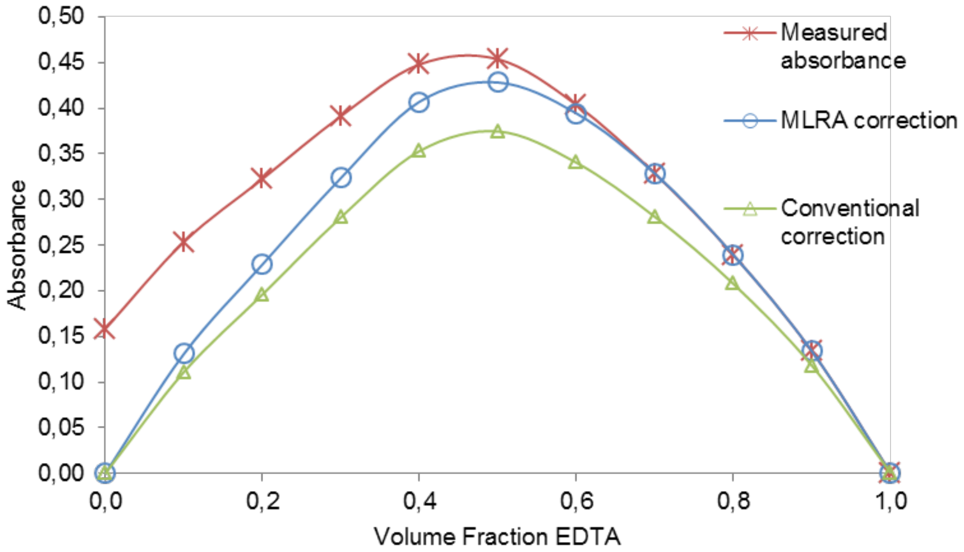
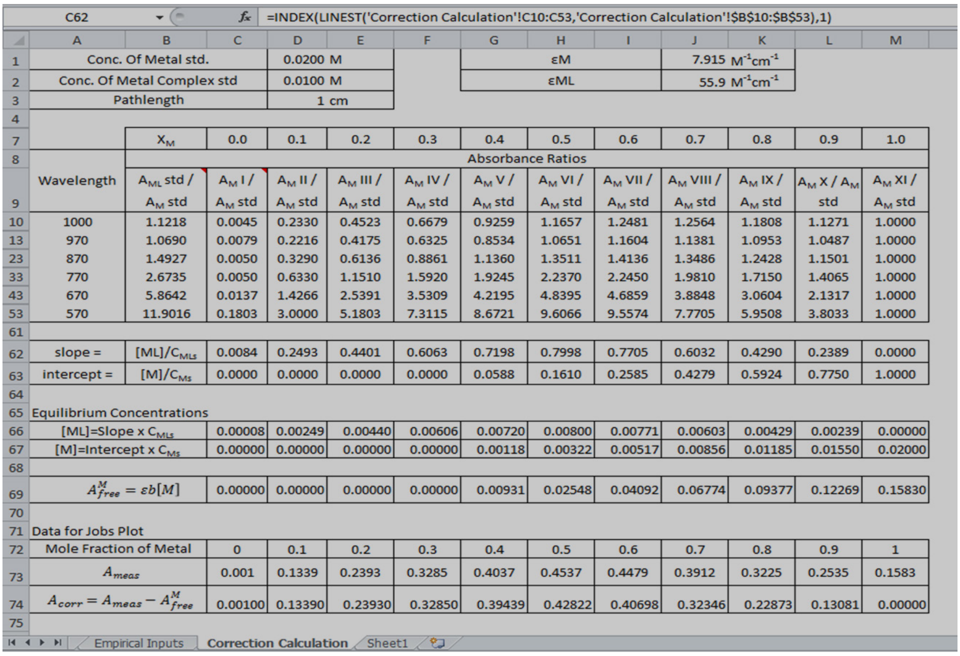


Figure 3. Jobs plot of Cu-EDTA system for the measured absorbance, the MLRA absorbance correction and conventional absorbance correction

$$A'_{corr} = A_m - \epsilon_{Cu^{2+}} b\, C_1 \tag{3}$$

The corrected absorbance from Eq. (3) was then used to construct the Job's plot. For comparison both correction methods were applied on the same data set and the absorbance data obtained from the two correction methods are shown in Table 2. The conventional correction method was studied at the λ_{max} of the complex.



C62 =INDEX(LINEST('Correction Calculation'!C10:C53,'Correction Calculation'!$B$10:$B$53),1)

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Conc. Of Metal std.			0.0200 M			εM			$7.915\ M^{-1}cm^{-1}$			
2	Conc. Of Metal Complex std			0.0100 M			εML			$55.9\ M^{-1}cm^{-1}$			
3	Pathlength			1 cm									
4													
7		X_M	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
8		Absorbance Ratios											
9	Wavelength	A_{ML} std / A_M std	A_M I / A_M std	A_M II / A_M std	A_M III / A_M std	A_M IV / A_M std	A_M V / A_M std	A_M VI / A_M std	A_M VII / A_M std	A_M VIII / A_M std	A_M IX / A_M std	A_M X / A_M std	A_M XI / A_M std
10	1000	1.1218	0.0045	0.2330	0.4523	0.6679	0.9259	1.1657	1.2481	1.2564	1.1808	1.1271	1.0000
13	970	1.0690	0.0079	0.2216	0.4175	0.6325	0.8534	1.0651	1.1604	1.1381	1.0953	1.0487	1.0000
23	870	1.4927	0.0050	0.3290	0.6136	0.8861	1.1360	1.3511	1.4136	1.3486	1.2428	1.1501	1.0000
33	770	2.6735	0.0050	0.6330	1.1510	1.5920	1.9245	2.2370	2.2450	1.9810	1.7150	1.4065	1.0000
43	670	5.8642	0.0137	1.4266	2.5391	3.5309	4.2195	4.8395	4.6859	3.8848	3.0604	2.1317	1.0000
53	570	11.9016	0.1803	3.0000	5.1803	7.3115	8.6721	9.6066	9.5574	7.7705	5.9508	3.8033	1.0000
61													
62	slope =	$[ML]/C_{MLs}$	0.0084	0.2493	0.4401	0.6063	0.7198	0.7998	0.7705	0.6032	0.4290	0.2389	0.0000
63	intercept =	$[M]/C_{Ms}$	0.0000	0.0000	0.0000	0.0000	0.0588	0.1610	0.2585	0.4279	0.5924	0.7750	1.0000
64													
65	Equilibrium Concentrations												
66	$[ML]$=Slope x C_{MLs}		0.00008	0.00249	0.00440	0.00606	0.00720	0.00800	0.00771	0.00603	0.00429	0.00239	0.00000
67	$[M]$=Intercept x C_{Ms}		0.00000	0.00000	0.00000	0.00000	0.00118	0.00322	0.00517	0.00856	0.01185	0.01550	0.02000
68													
69	$A^M_{free} = \varepsilon b[M]$		0.00000	0.00000	0.00000	0.00000	0.00931	0.02548	0.04092	0.06774	0.09377	0.12269	0.15830
70													
71	Data for Jobs Plot												
72	Mole Fraction of Metal		0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
73	A_{meas}		0.001	0.1339	0.2393	0.3285	0.4037	0.4537	0.4479	0.3912	0.3225	0.2535	0.1583
74	$A_{corr} = A_{meas} - A^M_{free}$		0.00100	0.13390	0.23930	0.32850	0.39439	0.42822	0.40698	0.32346	0.22873	0.13081	0.00000
75													

Figure 4. Screen shot of spreadsheet used to perform the required calculations

Table 2. Comparison of absorbance values determined using the correction methods

Formal Conc. (M)	Volume fraction Cu^{2+}	$A_{corr} = A_{meas} - \epsilon_M b C_M$	$A_{corr} = A_{meas} - \epsilon_M b[M]$	% ΔA_{corr}
0	0	0.001	0.001	0
0.002	0.1	0.11807	0.1339	-11.8
0.004	0.2	0.20764	0.2393	-13.2
0.006	0.3	0.28101	0.3285	-14.4
0.008	0.4	0.34038	0.3943	-13.7
0.01	0.5	0.37455	0.4282	-12.5
0.012	0.6	0.35292	0.4069	-13.3

0.014	0.7	0.28039	0.3234	-13.3
0.016	0.8	0.19586	0.2287	-14.4
0.018	0.9	0.11103	0.1308	-15.1
0.02	1.0	0	0	0

The Job's plots from the uncorrected data, the conventionally corrected and alternate methods are shown in Fig. 3. The corrected absorbance at the two extremes of the series were found quite similar for the two correction methods, but the difference was significant, ranging from 10 to 15%, for the rest of the series. An important educational aspect of method of continuous variation is the determination of equilibrium constant for the system under study. The equilibrium calculations rely on the absorbance values obtained from the plot. If the absorbance corrections are based on formal concentrations it would propagate huge error, shown in Table 2, and the analysis would yield erroneous values of equilibrium constant.

Conclusion

Pedagogically this article is intended to solve a problem related to solution equilibria by integrating multiple concepts used in undergraduate chemistry education. Moreover, the application of Multiwavelength-Linear-Regression-Analysis for the absorbance correction of Job's method came out as an alternate correction method which is reliable as compared to the conventional methods. This experiment practically shows the difference between formal and equilibrium concentrations and can also serve as an example to elaborate the concept in chemistry laboratory.

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🖂 **Dr. Agha Arslan Wasim**
Department of Chemistry
University of Karachi
75270 Karachi, Pakistan
E-mail: arslan.wasim@uok.edu.pk

🖂 **Prof. Muhammad Nasiruddin Khan**
Department of Chemistry
University of Karachi
75270 Karachi, Pakistan
E-mail: nasiruk@uok.edu.pk

🖂 **Dr. Muhammad Farooq Wahab**
Department of Chemistry and Biochemistry
University of Texas at Arlington
Arlington, TX, USA
E-mail: farooq.w@gmail.com

🖂 **Dr. Saba Fazal-ur-Rehman**
Department of Chemistry
University of Karachi
75270 Karachi, Pakistan
E-mail: saba@uok.edu.pk