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A SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION CO-EDTA COMPLEXES

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Abstract

A precise quantification of Co(II)EDTA complex is required to develop bioremediation approaches for Co(III)EDTA complex that is generated in various industrial processes. It is cumbersome to quantify Co(II)EDTA in a mixture of Co(II)EDTA and Co(III)EDTA by normal UV-visible spectrophotometric methods as both the complexes absorb significantly at 490 nm, which is the λ_{\max} of Co(II)EDTA. Whereas other sophisticated techniques such as gas chromatography, atomic absorption spectrophotometer, inductively coupled plasma-atomic emission spectroscopy can measure individual species of Co(II)EDTA or Co(III)EDTA when present alone but are ineffective to measure the Co(II)EDTA precisely when it is mixed with Co(III)EDTA. Hence, an attempt has been made to develop a spectrophotometric method for its quantification. This paper describes the development of a simple and economic dual wavelength spectrophotometric method for the determination of Co(II)EDTA in a mixture of Co(II)EDTA and Co(III)EDTA complexes. The wavelength pair, 490-580 nm was optimized for the measurement of absorbance for this dual wavelength method of determination of Co(II)EDTA in the presence of Co(III)EDTA.

Key words: Spectrophotometry, Dual wavelength method, Cobalt-EDTA complex.

Introduction

Co(III)EDTA is toxic, thermodynamically more stable and soluble compared to Co(II)EDTA. Reduction of Co(III)EDTA to Co(II)EDTA is an ideal method for Co(III)EDTA bioremediation (Ogino and Ogino, 1983; Gorby *et al.*, 1998; Jardine *et al.*, 2002, Krumholz *et al.*, 2003). Maximum spectrophotometric absorbance values (λ_{\max}) of Co(II)EDTA and Co(III)EDTA complexes are at 490 and 535 nm respectively (Hau *et al.*, 2008). In some of the earlier reports, reduction of Co(III)EDTA was monitored by measuring the decrease in the absorbance of Co(III)EDTA at 535 nm (Hau *et al.*, 2008). Since Co(II)EDTA also absorbs significantly at 535 nm, the estimation of Co(III)EDTA by measuring the decrease in absorbance at 535 nm does not give a correct quantification. Therefore, to monitor the reduction of Co(III)EDTA, a better method for the estimation of either Co(II)EDTA or Co(III)EDTA in a mixture of Co(II)EDTA and Co(III)EDTA complexes is mandatory. Individual species of Co(II)EDTA or Co(III)EDTA when present alone can be measured using various methods such as UV-visible absorption spectrophotometer (Flaschka and Barnard, 1976), gas chromatography (Rudling 1972) or atomic absorption spectrophotometer (Jardine *et al.*, 1993). However, the above said methods are ineffective to measure

the Co(II)EDTA precisely when it is mixed with Co(III)EDTA. Taylor and Jardine designed a method to quantify Co(II)EDTA and Co(III)EDTA mixtures using ion chromatography (Taylor and Jardine, 1995). Even though, ion-chromatographic method is an effective method in the separation and quantification of Co(II)EDTA from the mixture of Co(II)EDTA and Co(III)EDTA, it needs conductivity and UV-Visible detectors besides the requirement of more expensive ion-exchange columns. In the present study, a simple and economic dual wavelength spectrophotometric method was developed for the determination of Co(II)EDTA in the presence of Co(III)EDTA and the developed method could be effectively utilized for monitoring the reduction of Co(III)EDTA during the bioremediation studies.

Experimental

All the reagents used were AR/GR grade. EDTA was procured from Merk India Ltd. CoCl₂, sodium acetate, ethanol, hydrogen peroxide etc. were procured from H. Chandamal & Co, India. Chemito make UV-Visible double beam spectrophotometer was used for the absorbance measurements.

Preparation of Co(II)EDTA and Co(III)EDTA

Co(II)EDTA and Co(III)EDTA complexes were prepared by different methods. Co(II)EDTA was prepared by adding equal molar (0.5 M) amount of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ and EDTA solutions. Co(III)EDTA was prepared based on the method of Taylor and Jardine (1995). A solution of 2 g of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 3.2 g of Na_2EDTA and 7.2 g of sodium acetate in 15 ml of distilled water was boiled and 3.2 ml of 7% H_2O_2 (oxidizing agent) was added to the solution with continuous stirring. The solution was cooled to room temperature and 10 ml of 100% ethanol was added slowly with continuous stirring. The precipitated crystals were washed thrice with 100% ethanol and dried in open air. These crystals were dissolved in a small volume of double distilled water, re-crystallized in 100% ethanol and washed twice in ethanol. The re-crystallized crystals were dried at low temperature (40 °C) until all ethanol evaporated and the crystals were stored at 4 °C.

Spectrophotometric procedure

Different combinations of wavelength pairs viz. 490-535, 490-580, 510-535 and 510-550 nm were used to measure the absorbance of Co(II)EDTA and Co(III)EDTA complex mixture for arriving at a suitable dual wavelength pair for the quantitative estimation of Co(II)EDTA. Three different sets of Co-EDTA complexes viz. 1 mM, 5 mM and 10 mM were used for the calibration study. In each set, different calibration standards containing varying concentrations of Co(II)EDTA was prepared. Concentration of Co(II)EDTA was increased from 0-1, 0-5 and 0-10 mM, whereas the concentration of Co(III)EDTA was decreased from 1-0, 5-0 and 10-0 mM, respectively in the above three sets of Co-EDTA complexes so that the sum of the concentrations of Co(II)EDTA and Co(III)EDTA in the individual standards in each set remained constant at 1, 5 and 10 mM respectively. Absorbance of the standard solutions was measured using 1 cm quartz cell against double distilled water as blank.

Results and Discussion

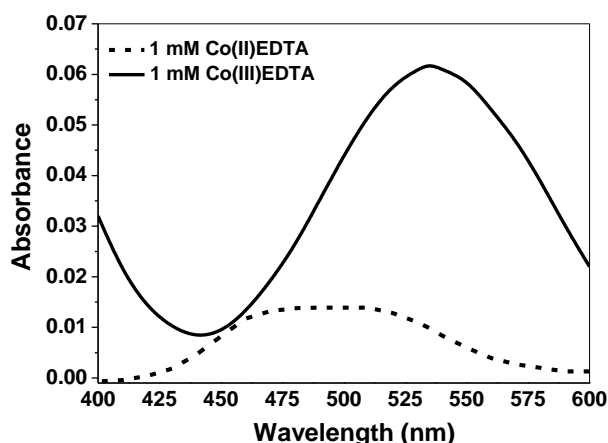


Fig. 1: Absorption spectrum of Co(II)EDTA and Co(III)EDTA in the visible range.

The absorption spectra of 1 mM concentration of Co(II)EDTA and Co(III)EDTA are given in figure 1. They absorb maximum at 490 and 535 nm (λ_{max}) respectively. From the spectra, it is clear that their individual quantification in the complex mixture is not possible by measuring the absorbance at their λ_{max} values as both the complexes absorb significantly at the two wavelengths. In certain cases, to avoid interference from other species, absorbance measurement can be made at other than λ_{max} region also. But, in the case of Co(II)EDTA and Co(III)EDTA, as both of them have significant absorbance in the region 400 – 600 nm, a single wavelength cannot be chosen for the estimation of Co(II)EDTA or Co(III)EDTA. However, the quantification of Co(III)EDTA may be possible at less than 400 nm as Co(II)EDTA does not absorb significantly at this region. This way, indirect quantification of Co(II)EDTA may be possible provided the initial concentration of Co(III)EDTA should be known. But, for the estimation of Co(II)EDTA at any point of time in the presence of Co(III)EDTA, a separate method is must. Hence, dual wavelength method is attempted for its estimation (Bhattacharyya and Kundu, 1971). It is known that if the absorbance values of two spectra overlapping species in dilute solutions are additive at two wavelengths, dual wavelength method can be applied for the estimation of the species of interest by properly selecting the wavelength pair. The pre-requisite for dual wavelength method is the selection of two such wavelengths where the interfering species shows almost same absorbance whereas the species of interest shows significant difference in absorbance. Since the absorbance values are additive at the selected two wavelengths, the concentration of Co(II)EDTA can be found out from absorbance measurements made at two separate wavelengths. If the ratios of molar absorptivities at two wavelengths, λ_1 and λ_2 are α and β for Co(III)EDTA and Co(II)EDTA respectively, then

$$A_{\lambda_1} - \alpha A_{\lambda_2} = \text{Co(II) EDTA} \times \epsilon_{\lambda_1} \left(1 - \alpha/\beta\right) \quad \text{-----(1)}$$

Where, A_{λ_1} and A_{λ_2} are the total absorbance at λ_1 and λ_2 nm respectively, and ϵ_{λ_1} is the molar absorptivity of Co(II)EDTA at λ_1 . A plot of $A_{\lambda_1} - \alpha A_{\lambda_2}$ Vs concentration of Co(II)EDTA should give a straight line passing through the origin.

Then,

$$\text{The concentration of Co(II)EDTA} = \frac{(A_{\lambda_1} - \alpha A_{\lambda_2})}{\text{slope}} \quad \text{----- (2)}$$

The ratio of the molar absorptivity of Co(III)EDTA at two different wavelengths (α) was calculated from the absorbance as below:

$$\alpha = \frac{\epsilon_{1\lambda_1}}{\epsilon_{1\lambda_2}} \quad \text{-----(3)}$$

$$\alpha = \frac{\text{absorbance of Co(III)EDTA at } \lambda_1 (A_{\lambda_1})}{\text{concentration of Co(III)EDTA} \times \text{Pathlength}} \bigg/ \frac{\text{absorbance of Co(III)EDTA at } \lambda_2 (A_{\lambda_2})}{\text{concentration of Co(III)EDTA} \times \text{Pathlength}}$$

$$= \left(\frac{\text{absorbance of Co(III)EDTA at } \lambda_1 (A_{\lambda_1})}{\text{concentration of Co(III)EDTA} \times \text{Pathlength}} \right) \times \left(\frac{\text{concentration of Co(III)EDTA} \times \text{Pathlength}}{\text{absorbance of Co(III)EDTA at } \lambda_2 (A_{\lambda_2})} \right)$$

$$\alpha = \frac{\text{absorbance of Co(III)EDTA at } \lambda_1 (A_{\lambda_1})}{\text{absorbance of Co(III)EDTA at } \lambda_2 (A_{\lambda_2})} \text{-----(4)}$$

Where, ϵ_1 is the molar absorptivity of Co(III)EDTA.

We started the analysis by using their λ max values 490 nm (λ_1) and 535 nm (λ_2) respectively of Co(II)EDTA and Co(III)EDTA as the wavelength pairs for the absorbance measurements. Significant error was observed in the estimated values. Hence, more wavelength pairs within the spectra overlapping region viz. 490-535, 490-580, 510-535 and 510-550 nm were examined for the dual wavelength method of calibration. In all the pairs, λ_1 is the lower wavelength and λ_2 is the higher wavelength.

Chi-square test was performed to evaluate the accuracy of the method for the estimation of Co(II)EDTA in a mixture of different combinations of known concentrations of Co(II)EDTA and Co(III)EDTA. Ratio of molar absorptivity (α) at λ_1 and λ_2 was calculated for all the above four wavelength pairs and the value of ($A_{\lambda_1} - \alpha A_{\lambda_2}$) was plotted against the concentration of Co(II)EDTA. Linearity and the slopes of the calibration curve obtained for each wavelength

pair were evaluated. The objective of carrying out the absorbance measurements at different combinations of wavelengths was to get constant slope at different concentration ranges of Co(II)EDTA in the presence of varying concentration of Co(III)EDTA, thereby to optimize the wavelength pair for the estimation with the least error possible.

Fig. 2 and Table 1 show that when the measurement was done at the wavelength pair, 490-580 nm, the slope of the calibration curve was not significantly affected in the presence of different concentration ranges of Co(II)EDTA and Co(III)EDTA, whereas, the other double wavelength combinations; 490-535, 510-535 and 510-550 nm showed significant variation in the slope for different concentrations of Co(II)EDTA as shown in Table 1. Also, in the case of 490-580 nm pair, the α -values were close to one viz. 0.90, 0.90 and 0.92 compared to other wavelength combinations.

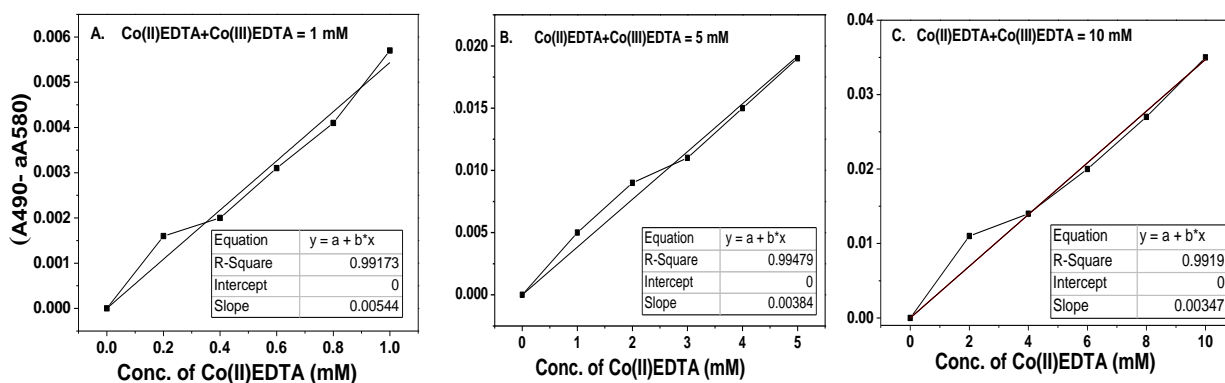


Fig. 2: Calibration curves of 0-1, 0-5 and 0-10 mM of Co(II)EDTA.

Table 1: Slopes of the calibration curves of Co(II)EDTA and the ratios of molar absorptivity (α) of Co(III)EDTA obtained with different wavelength pairs for different concentration range.

Combinations of double wavelengths (nm) ($\lambda_1:\lambda_2$)	Slope of calibration curve at different conc. of Co(II)EDTA				α -values at different conc. of Co(III)EDTA	
	0-1 mM	0-5mM	0-10 mM	1 mM	5 mM	10 mM
	(490:535)	0.0050	0.0145	ND	0.56 ± 0.024	0.60 ± 0.019
(490:580)	0.0054	0.0038	0.0035	0.90 ± 0.027	0.90 ± 0.032	0.92 ± 0.02
(510:535)	0.0027	0.0036	0.0009	0.84 ± 0.017	0.86 ± 0.015	0.89 ± 0.016
(510:550)	0.0032	0.0022	0.0016	0.88 ± 0.022	0.90 ± 0.012	0.98 ± 0.021

*ND= Not determined

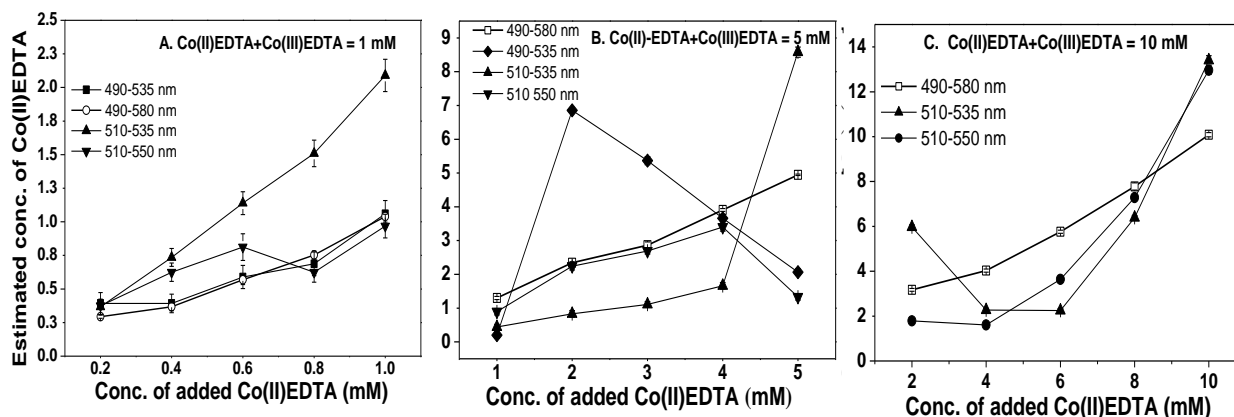


Fig. 3: Comparison of estimated concentration of Co(II)EDTA using the wavelength pair 490-580 nm and the actual concentration of Co(II)EDTA.

Fig. 3 show that the estimated concentration values of Co(II)EDTA, using the wavelength pair 490-580 nm, were very close to the added concentration of Co(II)EDTA in three different concentration ranges of Co(II)EDTA and Co(III)EDTA. On the other hand, for the other wavelength combinations; 490-535, 510-535 and 510-550 nm, estimated concentration of Co(II)EDTA varied significantly from the actual added concentration of Co(II)EDTA. The results were tested with chi-square test and in the case of 490-580 nm combination; the observed p values were 0.93, 0.94 and 0.98 respectively for 1, 5 and 10 mM of Co-EDTA. While for the other dual wavelength combinations, a consistent value of Chi-square could not be obtained. This shows that 490-580 nm combination is the ideal wavelength pair and hence can successfully be used to quantify Co(II)EDTA in a mixture of both the complexes. This is due to the significant difference in absorbance for the species of interest, (Co(II)EDTA) and almost same absorbance for the interfering species (Co(III)EDTA) at 490 and 580 nm compared to other wavelength pairs. Thus, the concentration of Co(II)EDTA can be obtained by using the equations (2) and (4), where λ_1 and λ_2 are 490 and 580 nm respectively.

It was observed that the interference of Co(III)EDTA reduced the accuracy of the measured result. The accuracy of this dual wavelength method for the analysis of Co(II)EDTA was found to be increased with the decrease in the concentration of Co(III)EDTA.

Conclusion

This paper establishes a very simple and economical dual wavelength spectrophotometric method for the determination of Co(II)EDTA in a mixture of Co(II)EDTA and Co(III)EDTA complexes. The wavelength pair, 490-580 nm was found to be the ideal one for the measurement of Co(II)EDTA in the presence of Co(III)EDTA. This method could successfully be used for the determination of Co(II)EDTA during the bioremediation studies of Co-EDTA complexes.

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