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Determination of low concentrations by spectrophotometry: Uranium–thiocyanate system

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Abstract. Errors in the determination of low concentrations by spectrophotometry are investigated with the uranium–thiocyanate system as an example. The reagent blank has significant absorption and measurements are made at 375 nm instead of the λ_{max}.

The error in the intercept of the calibration curve is an important factor in such measurements and the errors involved in the estimation of 1 μg/ml (normal working range 4–40 μg/ml) are studied. It is shown that both random and systematic errors associated with the intercept are responsible for observed errors. The two types of errors are resolved by ANOVA (analysis of variance). The error in the measurement of a single value is estimated and compared with measured values for different calibration ranges.

It is seen that two factors predominantly influence the error in the measured concentration – the variance from regression and the closeness of the measured value to the mean of the calibration range.

Keywords. Low concentration measurement; spectrophotometry; uranium–thiocyanate system; analysis of variance; errors in measurement.

1. Introduction

Spectrophotometric estimation involves the use of a calibration curve (generally linear) to estimate the concentration of an unknown. The characteristics of the calibration curve are determined by linear regression on the absorbance measurements of a finite number of standards. Decision and detection limits based on the calibration curve have been reported (Currie 1968; Hubaux and Vos 1970; Long and Winefordner 1983). The propagation of errors of calibration and measurement of absorbance for the unknown has been used to arrive at the error associated with the value obtained for the unknown. The detection limits, being defined in probabilistic terms, are not easy to verify in practice. Further in an analytical situation one is concerned with the problem of whether a given concentration can be determined and, if so, the error in such a measurement. A methodology has been developed in this paper to provide a solution to this problem.

The uranium–thiocyanate system, which is the example chosen in this study, is a system in which the reagent absorbance is comparable to that of the complex at λ_{max}; hence a different wavelength is used for the estimation. Even at this wavelength the

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reagent has significant absorption and this is a limiting factor in this method. The lower limit of determination of 4 μg/ml corresponds to an absorbance of $\sim 0{\cdot}08$. It should be possible to go down to levels lower by a factor of 10 or so. Hence it was of interest to investigate whether lower concentrations can be determined and to estimate the errors involved.

2. Theoretical considerations

The estimation of an unknown concentration X from the measured absorbance Y is based on the relationship:

$$Y = a + bX, \tag{1}$$

where a is the intercept and b the slope of the calibration line. These values are obtained from n standards with (X_i, Y_i) denoting the values of the ith standard. The values of a and b are calculated by linear regression (Snedecor and Cochran 1967, p. 135; Laitinen and Harris 1975). Two quantities derived from linear regression are pertinent to the present study

$$s_{y,x}^2 = \frac{\Sigma(Y_i - a - bX_i)^2}{(n-2)} \tag{2}$$

and

$$s_a = s_{y,x}\left\{\frac{1}{n} + \frac{\bar{X}^2}{\Sigma(X_i - \bar{X})^2}\right\}^{1/2} \tag{3a}$$

$$= s_{y,x}\left\{\frac{\Sigma X_i^2}{n\Sigma(X_i - \bar{X})^2}\right\}^{1/2} \tag{3b}$$

$$= s_{y,x} \cdot F, \tag{3c}$$

where $s_{y,x}$ is the deviation from regression and s_a the standard deviation of the intercept which is related to $s_{y,x}$ by a factor given in (3a), rearranged in (3b) and represented by F in (3c). The significance of this is that the factor is purely a ratio which does not depend on the absolute values of concentrations chosen but on the dispersion of values around the mean $\bar{X}$. Since s_a changes as the square root of n, the number of standards beyond 5 to 10 is not likely to bring about a significant change in the value of s_a. The value of s_a will largely determine the feasibility of estimating low concentrations.

3. Experimental

The determination of uranium is based on absorbance measurements at 375 nm of the uranium thiocyanate complex in a medium consisting of 75% standard alcoholic ammonium thiocyanate, 20% ethylacetate and 5% stannous chloride. The measurements are carried out at 375 nm though the λ_{max} is 340 nm. The working range is 4 to 40 μg/ml. The method (Desesa and Nietzel 1954) has been in use in our laboratory for a considerable period of time and was used without modification.

3.1 *Reagents*

Ammonium thiocyanate – 650 g ammonium thiocyanate (AR grade) were added to

1 litre of a water-alcohol mixture (1:2). The solution is freshly prepared for each determination. Ethylacetate used was of AR grade. Stannous chloride – a 10% solution in 1 M hydrochloric acid was used. Uranium standard solution – 1 g uranium metal, after surface cleaning, is dissolved in nitric acid, evaporated to dryness and made up to 1 l with water to give the stock solution of 1 mg/ml. This solution is progressively diluted by factors of ten for different ranges.

3.2 *Colour development*

An aliquot of uranium solution is added to a 25 ml flask and volume made up with colour developing reagent consisting of a mixture of 75% by volume of ammonium thiocyanate reagent, 20% ethyl acetate and 5% stannous chloride. Absorbance measurements were carried out 30 minutes after colour development.

Absorbance measurements were made with a Shimadzu UV-240 recording spectrophotometer provided with an auto-flow cell. The cell is rinsed with the solution and filled automatically (without manual handling). Since the optical surface is not handled errors of measuring low values of absorbance are minimised. The instrument is zeroed with the reagent blank and solutions are measured after this. The absorbance values are measured automatically and values printed out. This eliminates operator bias in replicate measurements.

3.3 *Preliminary experiments*

The reproducibility of calibration constants was checked by carrying out calibration experiments using different concentration ranges and comparing the 95% confidence limits of slope and intercept obtained. The feasibility of determining concentrations in the range of 0·5–3·0 μg/ml was studied using a calibration curve in the range of 3·0–6·0 μg/ml. As the error of measurement was found to be of the order of 10%, it was decided to investigate in detail the measurement of concentrations of about 1 μg/ml.

3.4 *Errors of measurement*

Errors of measurement are expected to vary with the calibration range used. Hence three calibration ranges were chosen namely 0·5–1·0 (range 1), 5–10 (range 2), and 50–100 (range 3) μg/ml with 6 standards in each range. Six separate solutions were prepared for the sample (1 μg/ml) and absorbance measurements were carried out as mentioned above.

The calibration ranges were so chosen that the first two are below and above the concentration studied. By combining ranges 1 and 2 the sample concentration can be brought within this range and the effect of increasing the number of calibration points can also be studied.

The concentration values for the sample were calculated from the calibration constants calculated for each range by linear regression. The mean of the sample values and standard deviations were calculated and compared with the true value by the *t* test (Laitinen and Harris 1975). The *t* values were found to be significant in most of the cases, indicative of the systematic error present.

To see whether the trend is observed consistently, five sets of measurements were

Table 1. Calibration characteristics and values obtained for the samples.

	Set 1	Set 2	Set 3	Set 4	Set 5
Range 1					
Calibration (Values in OD units) @					
Slope *b*	17·71	21·62	20·29	56·28	26·57
Intercept *a*	−0·0476	−0·0353	0·0026	−0·0150	−0·0658
s_a	0·0019	0·0039	0·0024	0·0030	0·0040
$s_{y,x}$	$1·05 \times 10^{-3}$	$2·15 \times 10^{-3}$	$1·30 \times 10^{-3}$	$1·61 \times 10^{-3}$	$2·20 \times 10^{-3}$
Samples (Values in μg/ml)					
Mean	1·013	0·888	0·767	0·936	0·890
S.D.	0·046	0·068	0·073	0·057	0·073
t	0·67	−4·14**	−7·86**	−2·77*	−3·71*
Range 2					
Calibration (Values in OD units) @					
Slope *b*	21·63	19·14	18·23	26·17	21·45
Intercept *a*	−0·0597	−0·0266	−0·0160	−0·0166	−0·0687
s_a	0·0060	0·0124	0·0088	0·0054	0·0036
$s_{y,x}$	$3·28 \times 10^{-3}$	$6·77 \times 10^{-3}$	$4·81 \times 10^{-3}$	$2·95 \times 10^{-3}$	$1·98 \times ,0^{-3}$
Samples (Values in μg/ml)					
Mean	1·389	0·545	0·119	2·074	1·237
S.D.	0·038	0·077	0·081	0·122	0·090
t	25·21**	−14·49**	−26·73**	21·48**	6·42**
Range 3					
Calibration (Values in OD units) @					
Slope *b*	18·11	17·83	17·60	19·80	16·14
Intercept *a*	−0·0316	−0·0322	−0·0239	−0·0448	−0·0406
s_a	0·0131	0·0079	0·0087	0·0445	0·1492
$s_{y,x}$	$7·14 \times 10^{-3}$	$4·33 \times 10^{-3}$	$4·72 \times 10^{-3}$	0·0242	0·0811
Samples (Values in μg/ml)					
Mean	0·107	0·899	−0·326	4·165	−5·128
S.D.	0·045	0·082	0·084	0·162	0·120
t	−48·52**	−2·99**	−38·83**	47·91**	−124·83**
Range 1 + 2					
Calibration (Values in OD units) @					
Slope *b*	20·42	20·04	19·78	23·03	20·56
Intercept *a*	−0·0500	−0·0336	0·0037	0·0084	−0·0616
s_a	0·0012	0·0021	0·0017	0·0029	0·0011
$s_{y,x}$	$2·78 \times 10^{-3}$	$4·68 \times 10^{-3}$	$3·83 \times 10^{-3}$	$6·61 \times 10^{-3}$	$2·39 \times 10^{-3}$
Samples (Values in μg/ml)					
Mean	0·996	0·870	0·733	1·271	0·945
S.D.	0·040	0·073	0·074	0·140	0·094
t	−0·36	−6·14**	−12·43**	6·75**	−2·14*

Significance of *t* values * Significant at 5% level; ** Significant at 1% level; @ Except for slope.

carried out. The values obtained are summarised in table 1. The presence of systematic error was seen in most of the mean values for the five sets of measurements. Since the t values were high in some of the cases it was of interest to investigate the source of the error. Errors of this magnitude cannot be accounted for by the variations in the values of the slope; hence it was concluded that the source of error must be the variations in the value of the intercept. The magnitude of systematic error was estimated by one-way analysis of variance (one-way ANOVA–Snedecor and Cochran 1967, p. 258; Laitinen and Harris 1975) for the three ranges studied and the results are shown in table 2. The F values calculated (ratio of between-the-sets and within-the-set

Table 2. One way ANOVA of results from different calibration ranges.

	Sum of squares	Degrees of freedom	Mean square	F	S.D.@
Range 1					
Between	0·1918	4	0·0480	11·65**	0·085
Within	0·1029	25	$4{\cdot}171 \times 10^{-3}$		
Range 2					
Between	13·90	4	3·476	469·50**	0·760
Within	0·185	25	$7{\cdot}400 \times 10^{-3}$		
Range 3					
Between	276·3	4	66·83	5919·40**	3·34
Within	0·2824	25	0·0113		
Range 1 + 2					
Between	0·948	4	0·237	29·08**	0·195
Within	0·204	25	$8{\cdot}150 \times 10^{-3}$		

@ Standard deviation due to systematic error component (μg/ml); ** Significant at 1% level.

Table 3. Error estimate and observed value of error.

	Range 1	Range 2	Range 3	Range 1 + 2
Random selection of readings		(Values in μg/ml)		
1	0·113	0·727	3·34	0·173
2	0·120	0·761	3·24	0·502
3	0·136	0·831	3·37	0·269
Calculated from table 2	0·107	0·760	3·34	0·215
Calculated by propagation of errors@	0·103 to 0·306	0·193 to 0·659	0·454 to 9·24	0·073 to 0·189

@ Gives the minimum and maximum values calculated for the five sets. Calculations based on Long and Winefordner (1983).

variance) were highly significant. The systematic error component is comparable to the random error component in range 1 but higher in the other ranges. The systematic error component varied from set to set as revealed by the between-the-sets variance and assuming this variation to be a random variable, the standard deviation has been calculated as shown in table 2. The standard deviation for a single measurement is calculated by summing the variances due to random and systematic error components and this is given in table 3. The calculated value is compared with three sets of values estimated for the different ranges by choosing measured concentrations at random from each set. The estimate of error calculated from the propagation of errors is also included in table 3.

4. Results and discussion

The values of the intercept and its standard deviation are important factors which determine the feasibility of estimating low concentrations. The results in table 1 show the variations of these two from set to set and for different ranges within a set. If the intercept is regarded as equivalent to a blank value, three times its standard deviation will be the detection limit. Taking the slope to be approximately 20 ml/mg, the detection limits will be 0·6, 1, 2 and 1 μg/ml. However the results in table 1 show that the concentration level chosen is well within the range of determination especially for range 1.

The results of the analysis of variance appearing in table 2 highlight the presence of systematic error and its magnitude for the four ranges of calibration. The systematic error component varies from set to set as revealed by the ANOVA test and the variance due to this is calculated as shown in table 2. For ranges 2 and 3 this value is much higher than the random error (within-the-set variance). The principle of additivity of variances has been used to estimate the error for a single measurement and these are compared with the values estimated from three sets of measured concentrations chosen at random from each set. The error estimate based on propagation of errors is also included in table 3. The error estimate derived from analysis of variance agrees closely with the observed values. The better agreement seen in ranges 2 and 3 are essentially due to the predominance of systematic error component in these ranges. The propagation of error approach (Long and Winefordner 1983) gives a range of values since this is determined by the value of $s_{y,x}$ and it varies within each set. While the estimated values are in good agreement with the calculated values for the lower ranges the disagreement in higher ranges is essentially due to the higher values of $s_{y,x}$ observed in some of the sets. The source of the systematic error is most likely to be a corresponding error in the intercept as already seen.

The variation in the value of the intercept which gives rise to the variation in the systematic error component needs further consideration. The departure from a value of zero is due to the random errors of measurement as seen in the value of s_a. The observed values of intercept are far in excess of what can be accounted for by the value of s_a as seen by the t test (8 out of 20 values are significant at 1% level and 2 at 5% level). The presence of systematic error in the intercept can therefore be inferred. This cannot be attributed to the drift in the value of intercept as this will affect all the values and different concentration ranges equally. However the observation is that the magnitude

of the systematic error component and its variation are higher in the higher calibration range. The value of the intercept is derived from the mean values of Y, X from the relationship $a = \bar{Y} - b\bar{X}$. For example a lower value of b will lead to a higher value of intercept and a lower concentration value for the sample. This is seen to be the case with the results of range 3 sets III and V. The results of set IV for which the slope is higher show the opposite trend. In general the values of slope seem to be lower in range 3 though the reason for this is not clear.

In general the effect of systematic error is more readily seen in range 3. This can be explained as follows. If the value of sample concentration obtained using the true value of slope b_0 is given by

$$X_0 = \frac{(Y-a)}{b_0} = \frac{(Y-\bar{Y})}{b_0} + \bar{X}, \tag{4}$$

and that using the observed value of the slope by

$$X = \frac{(Y-\bar{Y})}{b} + \bar{X}, \tag{5}$$

so that

$$\Delta X = \frac{(Y-\bar{Y})}{b}\left(\frac{\Delta b}{b_0}\right), \tag{6}$$

where $\Delta X = X - X_0$ and $\Delta b = b_0 - b$.

Hence the error in the measured concentration will depend on its closeness to the mean value of the calibration range and also the error in the slope. The difference between Y and $\bar{Y}$ values progressively increases as one goes from range 1 to 3 corresponding to the concentration of 1 μg/ml for the sample and mean values of 0·75, 7·5 and 75 μg/ml for the three ranges. It is significant that the combined range of 1 + 2 has not resulted in a significant reduction in systematic error. The other factor which contributes to the error in X is the value of Δb which is determined by the value of $s_{y,x}$. This is also higher for range 3. Hence the error in the measurement of low concentrations is determined by the closeness of the mean value of the calibration range to the measured value and the standard deviation from regression. Viewed from this point, the determination of low concentrations appears to be no different from higher values, provided one can construct a calibration curve with reasonable accuracy with calibration points around the sample value.

The intercept corresponds to zero concentration which is farthest from the mean value. Both random and systematic errors are the highest, (3a) and (6), for the intercept. Hence detection limits based on s_a are likely to be too high.

5. Conclusion

The method of study developed in this paper brings out the contribution of the two main sources of error in the measurement of low levels of concentration. The uranium thiocyanate system has been chosen as an example because of the contribution of the reagent towards measured absorption and this is a limiting factor. It has been possible to lower the level of determination of uranium by a factor of four with good accuracy. In cases where systematic error of the intercept is negligible it is possible to optimise

the calibration range by a choice of calibration points such that the factor F (3c) is minimum.

The methodology developed in this study can be applied to any system where low levels are determined through a calibration curve.

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References

Currie L A 1968 *Anal. Chem.* **40** 586

Desesa M A and Nietzel O A 1954 Spectrophotometric determination of uranium with thiocyanate, ACCO-54

Huhaux A and Vos G 1970 *Anal. Chem.* **42** 849

Laitinen H A and Harris W E 1975 in *Chemical analysis* Int. Student Edn (Tokyo: McGraw Hill-Kogakusha) p. 531

Long G L and Winefordner J D 1983 *Anal. Chem.* **55** 712 A

Snedecor and Cochran 1967 in *Statistical methods* 6th edn (Oxford: IBH) pp. 135, 258