화학계량학적 방법을 사용한 Triton X-100이 함유된 1-(2-Thiazolylazo)-2-Naphthol을 사용한 구리, 니켈과 아연의 동시 분광광도법적 정량

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Simultaneous Spectrophotometric Determination of Copper, Nickel, and Zinc Using 1-(2-Thiazolylazo)-2-Naphthol in the Presence of Triton X-100 Using Chemometric Methods

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INTRODUCTION

It is generally agreed that river is the main source of drinking water in Malaysia, therefore assessment and monitoring of river water quality for effective and reliable management options is undoubtedly essential. For this purpose, the effluent quality of any discharge from a sewage treatment process to inland water and catchments areas located upstream of drinking water supply off-takes have to meet the minimum requirements of the Malaysian Environmental Quality Act 1974. The limits set down by the Environmental Quality (Sewage Industrial Effluent) Regulations, 1979 are not only regarding physical parameters but also chemical measurements such as oxygen related parameters and metal contents are included.¹,²

Uluozlu et al., (2007), suggested that metals could be possibly classified as potentially toxic (arsenic, cadmium, lead, mercury, etc.), probably essential (cobalt, nickel, vanadium,) and essential (copper, iron, manganese, zinc).³ The toxic elements can be very harmful even at low concentration if ingested over a long period of time. Essential metals could also produce toxic effects if their intake was excessive.⁴ They could be accumulated in the water supply through human activities, such as industrial and consumer wastes. Commercial processes, like mining, agriculture, manufacturing and the discarding of wastes in landfills are all common sources of metal contamination.⁵ Even rainwater with its acidic pH, could cause these compounds to leach into the surface and underground water supplies from the surrounding soil and rock.⁶,⁷ Due to these possibilities, the metals content in our drinking water has to be monitored, in order to ensure the safety as well as quality.

In order to quantify the metal concentration in water, this is most often accomplished via either spectrometry or electrochemical technique. As an example, flame atomic absorption spectrometry (FAAS) is relatively low cost with good analytical performance. However due to the moderate limit of detection and matrix effects, separation or pre-concentration steps are needed for determination of metals at low concentration.⁸ Several applications of these methods have been reported.⁹,¹⁰ Graphite furnace atomic absorption spectrometry (GFAAS) could provide better detection limits, but there are relatively higher interference from the matrix and requiring a longer analysis time. Furthermore both methods only afford assay of a single element at one time. The most excellent technique for elemental analysis nowadays is the inductively coupled plasma mass spectroscopy (ICPMS). It can simultaneously screen up to almost all elements in a single sample run in less than a minute, with high precision and excellent detection limits. However, all these have to be compromised with very high capital and operation costs as well as skilled personnel.

On the other hand, spectrophotometric methods based on various chromogenic reagents offer more economic ways for rapid determination of metal cations compared to techniques that have been mentioned.¹² This is the instrumental technique of choice in industrial laboratories, owing mainly to its simplicity and often demanding low cost equipment.¹³ However the drawback for performing simultaneous determinations is the high degree of spectral overlapping of these constituents. In order to perform calibration, a preliminary treatment such as addition of suitable masking agents or carrying out extraction steps is needed, which definitely makes the operation laborious.¹⁴ Nevertheless, with the aid of chemometric tools, which eases the interpretation of complicated spectra, the problem is solved. Multivariate calibrations allow extraction of quantitative information from such systems without major pretreatment.¹⁵ In other words, employing chemometric methods enable compensation of interference, where multivariate calibration is performed by ignoring the concentration of all other components except the analyte of interest.¹⁶,¹⁷ For instance, partial least square (PLS), principal component regression (PCR) and artificial neural networks (ANNs) have been successfully applied for simultaneous determinations of metals in different matrices.¹⁸,¹⁹,²⁰ In 2009, Goordazi et al. have demonstrated application of principal component analysis-adaptive neuro-fuzzy inference systems for the simultaneous determina-
tion of ternary mixtures in water samples.\textsuperscript{21}

In spectrophotometric methods, complexing agents such as thiazolylazo dye have been successfully employed in simultaneous metal determinations in conjunction with chemometric methods.\textsuperscript{22} These compounds have attracted much attention not only by virtue that they are sensitive chromogenic reagents for spectrophotometric, liquid chromatography and extraction-photometric (including solid phase, liquid-liquid and cloud point extraction) determinations of many metal ions, but also because of their fastness of colour formation and low price.\textsuperscript{23}

The significant drawback for most of these compounds and their complexes are only partly soluble or insoluble in water depending on the pH.\textsuperscript{24} However, surfactants and buffer have been conveniently used to enable the measurement in an aqueous medium.\textsuperscript{25,26} The compounds that are widely used includes 1-(2'-thiazolylazo)-2-naphthol (TAN) due to its ability to form azo-metal chelates with various cations.\textsuperscript{27} A number of applications have been proposed regarding the determination of transition metals such as copper, iron, manganese, nickel, zinc etc. using TAN.\textsuperscript{28,29,30,31}

In the present work, we made use of commercially available TAN in the investigation and development of rapid spectrophotometric methodology for the simultaneous determination of copper, nickel and zinc ions in water. A simple method is proposed, by making use of digitized spectroscopic data, commercial software and robust multivariate calibration which allow fast and sensitive determination of these components in a mixture, thus avoiding preliminary treatment.

**EXPERIMENTAL**

**Instrumentation**

Spectrophotometric acquisitions from 200.0 to 800.0 nm were carried out using a Cary 50 UV-Visible spectrophotometer (Varian), with 10.0 mm path length quartz cuvette (Hellma), at a scan rate of 4800 nm/min. The spectra were digitized in 1.0 nm intervals by Cary WinUV, Ver. 3.00 software and exported as SPC file format for subsequent quantitative data evaluations using the CAMO® Unscrambler® V. 9.7. A CyberScan pH 1100 (Eutech) meter was used for pH measurements at 25 °C.

**Reagents and Standard Materials**

All chemicals used in this work were at least of analytical grade. Deionized water was obtained using ELGA® PURELAB® UHQ II (> 18 MΩ cm\textsuperscript{-1} resistance). All glassware were cleaned by overnight soaking in dilute 10% nitric acid (w/v) then in deionized water prior to use.

1.00 mol dm\textsuperscript{-3} of ammonium acetate stock solution was freshly prepared using TraceSELECT\textsuperscript{®} ≥ 99.9999% (metals basis) quality salt from Fluka (Netherlands). Chromogenic reagent stock, 0.70 g L\textsuperscript{-1} TAN solution was prepared by dissolving the required amount of 1-(2-thiazolylazo)-2-naphthol puriss p.a. from Fluka (Japan) with LiChrosolv\textsuperscript{®} grade methanol from Merck (Germany). This ensures a sufficient reagent excess. Surfactant stock solution, Triton X-100 10.0% (v/v) was prepared by appropriate dilution of SigmaUltra Triton\textsuperscript{®} X-100 from Sigma-Aldrich (USA).

All calibration and validation standard solutions or synthetic mixtures were prepared by mixing 1.00 mL of ammonium acetate solution, 1.00 mL of 10.0% Triton X-100 and 1.00 mL of TAN solution with appropriate aliquots of the CertiPUR\textsuperscript{®} metal standard solutions (Merck) in a 25.00 mL volumetric flask. For the test set, aliquots of metal standard were substituted by 20.000 mL of tap water and spiked with known amount of desired metals.

**Experimental Design**

A 7-level factorial design was employed to figure out all the possible combinations for Cu\textsuperscript{2+}, Ni\textsuperscript{2+} and Zn\textsuperscript{2+} mixtures where concentration of each metal was varied between 0.050 ppm and 0.600 ppm to cover a satisfactory distribution in calibration range. Out of 343 total combinations, only 84 mixtures were randomly subjected to spectrophotometric analysis. The analyses were carried out in triplicates. As a result, a data matrix composed of 252 samples was obtained. The first 152 were considered as the calibration set for model estimation and the rest were
treated as validation set for confidence assessment. In order to statistically maximize the information in calibration matrix, blank samples and individual standards of Cu$^{2+}$, Ni$^{2+}$, and Zn$^{2+}$ ions with concentrations ranging from 0.050 ppm to 0.600 ppm were included.

**Estimation of figures of merit**

Estimation of analytical figures of merit (FOM) is necessary to quantify the quality of a given methodology or as a method of comparison, or used in verification. Analytical figures of merit such as sensitivity (SEN) that measures the changes in response as a function of the concentration of a particular analyte is given by:

$$\text{SEN} = \frac{1}{||b_k||}$$  \hspace{1cm} (1)

where $|| \cdot ||$ is the Euclidian norm of vector and $b_k$ is the vector of final regression coefficients appropriate for component $k$, which can be obtained by any multivariate method.  

On the other hand, analytical sensitivity ($\gamma$) can be defined in analogy to univariate calibration, as the quotient:

$$\gamma = \text{SEN} / ||\varepsilon||$$  \hspace{1cm} (2)

where $\varepsilon$ is a measure of the instrumental noise and it can be taken as an approximation to standard deviation of several blanks. The inverse of $\gamma$ ($\gamma^{-1}$) establishes a minimum concentration difference that is discernible by the method. In this way one can compare analytical methods regardless of the specific technique, equipment, and scale employed.

Different approaches exist for estimating the limit of detection (LOD) and quantification (LOQ) in multivariate calibration. The simplest one is derived by comparison with univariate methods. It is approximately three times the instrumental noise divided by the sensitivity for the parameter of interest for LOD and 10 times for LOQ. Estimations are based on the following expressions:

$$\text{LOD} = 3 \ ||\varepsilon|| \ ||b_k||$$  \hspace{1cm} (3)

$$\text{LOQ} = 10 ||\varepsilon|| \ ||b_k||$$  \hspace{1cm} (4)

**RESULTS AND DISCUSSION**

TAN reacted with Cu$^{2+}$, Ni$^{2+}$ and Zn$^{2+}$ instantaneously at room temperature to form coloured complexes (brown or red) in the presence of Triton X-100. The absorption spectra over 300.0 - 750.0 nm of the individual metal ion solutions for Cu$^{2+}$, Ni$^{2+}$ and Zn$^{2+}$ are almost similar, where they peaked around 491 nm due to excess chromogenic reagent TAN [42]. However, it could be observed that there are some variations in the visible region between yellow and red due to the characteristic of particular TAN-metal species as reported by Omar and Mohamed (2005). Consequently, the wavelength range from 550.0 to 700.0 nm with 1.0 nm intervals is highlighted as it provides the greatest amount of information about the various TAN-metal complexes (Fig. 1). However, the spectrum of each component overlaps each other, thus they cannot be determined directly using traditional calibration procedure without prior treatment. Due to this reason, multivariate calibration, which fully utilizes the selected spectrum range was adopted for simultaneous estimation of the concentration of each compound in the mixture without much laborious operation.

**PCR and PLS**

PCR and PLS are two well-known multivariate linear calibration methods in the field of chemometrics in general, and spectroscopy in particular. Both have been demonstrated as useful techniques...
Simultaneous Spectrophotometric Determination of Copper, Nickel, and Zinc

To quantitatively analyse spectra with increased matrix complexity, for this reason, PCR and PLS-2 were performed on the calibration set (228 × 151) to develop models for estimating Cu$^{2+}$, Ni$^{2+}$ and Zn$^{2+}$ in water. As a result, two optimized models were established and consequently validated with test set. The selection of optimal number of factors/PCs used to construct PLS or PCR model represents a decisive step in improving the prediction power of the methods. The criteria used for the selection of appropriate number of factor or principal components (PCs) involves selecting that model including the smallest number of factors/PCs that result in an insignificant difference between the corresponding root mean square error of calibration (RMSEC). Fig. 2 shows the variation of RMSEC as a function of the number of factors/PCs. In our case, 3 factors/PCs were found to be optimum for each compound by both PLS-2 and PCR methods. In spectroscopy, it is usually expected to get as many components as there are compounds present in the mixture in the case of non-highly overlapping system when standards are used during the calibration step.

The performance of the regression models was evaluated by standard errors of calibration/validation (SEC/P), RMSEC/P, the correlation between predicted and experimental values (R) as well as bias. The statistic parameters were summarised in Table 1. It can be seen that all $R^2$ values are in agreement with RMSEP for all the three compounds. According to Jha et al., (2005), a good model should have a low value of SEC and SEP, a high value of R, and a small difference between SEC and SEP. Large difference indicates either too many latent variables or PCs are used or ‘noise’ is modelled. The results obtained by PCR and PLS-2 methods using only 3 factors/PCs are quite similar to each other. This is clearly observed for both the loading (Fig. 3) and regression coefficients (Fig. 4). The results also demonstrate that the predicted concentrations are very close to the actual concentrations for each compound and this clearly reveal the validity of the calibration models. Based on the results, it can be said that there are no significant difference between both procedures.

Fig. 2. Plot of RMSEC vs. number of PCs/ factors for (a) PCR model (b) and PLS-2 model.

Fig. 3. First 3 loadings for both PLS-2 and PCR models.

Fig. 4. Both PLS-2 and PCR raw regression coefficients for each component using 3 factors/PCs.
Some conclusions can also be drawn from the loadings plot. The first factor/PC reveals that the loading peak around 583 nm relates to the peak of Zn complex (Fig. 1). The second component shows maximum loading at 599 nm, which corresponds to the maximum absorption of the Ni complex which suggests that TAN-Ni complex has the least overlapped spectrum. The third component most probably corresponds to the Cu complex because it describes around 45% of the variance of Cu2+, compared to less than 10% for Ni2+ and only 2% for Zn2+. Similar results are also observed in the regression coefficients plot where the plot is also observed to reflect the pure spectra pattern of each complex. This observation supports the sufficiency of 3 factors/PCs in describing the model.

### Figures of merit

Estimated FOM for Cu2+, Ni2+ and Zn2+ were also determined for PLS-2 and PCR models (Table 2).

### Table 1. Calibration and validation results for both PLS-2 and PCR models.

<table>
<thead>
<tr>
<th>No of Sample</th>
<th>PLS-2</th>
<th>PCR</th>
<th>PLS-2</th>
<th>PCR</th>
<th>PLS-2</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu (II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ni (II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn (II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>0.9940</td>
<td>0.9938</td>
<td>0.9984</td>
<td>0.9984</td>
<td>0.9973</td>
<td>0.9973</td>
</tr>
<tr>
<td>Offset</td>
<td>0.0013</td>
<td>0.0014</td>
<td>0.0003</td>
<td>0.0004</td>
<td>0.0006</td>
<td>0.0006</td>
</tr>
<tr>
<td>Correlation</td>
<td>0.9970</td>
<td>0.9969</td>
<td>0.9992</td>
<td>0.9992</td>
<td>0.9986</td>
<td>0.9986</td>
</tr>
<tr>
<td>R²</td>
<td>0.9940</td>
<td>0.9938</td>
<td>0.9985</td>
<td>0.9984</td>
<td>0.9973</td>
<td>0.9973</td>
</tr>
<tr>
<td>RMSEC</td>
<td>0.0166</td>
<td>0.0169</td>
<td>0.0082</td>
<td>0.0084</td>
<td>0.0106</td>
<td>0.0106</td>
</tr>
<tr>
<td>SEC</td>
<td>-2.206 × 10⁻⁸</td>
<td>-2.160 × 10⁻⁸</td>
<td>5.964 × 10⁻⁹</td>
<td>5.212 × 10⁻⁹</td>
<td>6.536 × 10⁻¹¹</td>
<td>-1.536 × 10⁻⁹</td>
</tr>
<tr>
<td>Explained Variance</td>
<td>99.404%</td>
<td>99.382%</td>
<td>99.848%</td>
<td>99.841%</td>
<td>99.727%</td>
<td>99.726%</td>
</tr>
</tbody>
</table>

### Table 2. Analytical figures of merit obtained using of PLS-2 and PCR.

<table>
<thead>
<tr>
<th>AFM</th>
<th>Cu (II)</th>
<th>Ni (II)</th>
<th>Zn (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>b₁</td>
<td>1.9469</td>
<td>1.9470</td>
<td>1.2367</td>
</tr>
<tr>
<td>c</td>
<td>0.0018</td>
<td>0.0019</td>
<td>0.0011</td>
</tr>
<tr>
<td>SEN</td>
<td>0.5136</td>
<td>0.5136</td>
<td>0.8119</td>
</tr>
<tr>
<td>γ⁻¹ / ppm</td>
<td>0.0036</td>
<td>0.0036</td>
<td>0.0013</td>
</tr>
<tr>
<td>LOD / ppm</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>LOQ / ppm</td>
<td>0.01</td>
<td>0.01</td>
<td>0.009</td>
</tr>
</tbody>
</table>

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Table 3. Intraday determination of Cu$^{2+}$, Ni$^{2+}$ and Zn$^{2+}$ in spiked tap water.

<table>
<thead>
<tr>
<th>Ion Species Spiked (ppm)</th>
<th>Found (ppm)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
<th>Found (ppm)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu (II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.100</td>
<td>0.103 ± 0.004</td>
<td>3.39</td>
<td>103 ± 4</td>
<td>0.103 ± 0.004</td>
<td>3.44</td>
<td>103 ± 4</td>
</tr>
<tr>
<td>0.300</td>
<td>0.309 ± 0.004</td>
<td>1.39</td>
<td>103 ± 1</td>
<td>0.310 ± 0.004</td>
<td>1.40</td>
<td>103 ± 1</td>
</tr>
<tr>
<td>0.500</td>
<td>0.526 ± 0.006</td>
<td>1.13</td>
<td>105 ± 1</td>
<td>0.526 ± 0.006</td>
<td>1.14</td>
<td>105 ± 1</td>
</tr>
<tr>
<td>Ni (II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.100</td>
<td>0.106 ± 0.002</td>
<td>1.71</td>
<td>106 ± 2</td>
<td>0.106 ± 0.002</td>
<td>1.75</td>
<td>106 ± 2</td>
</tr>
<tr>
<td>0.300</td>
<td>0.307 ± 0.002</td>
<td>0.76</td>
<td>102 ± 1</td>
<td>0.307 ± 0.002</td>
<td>0.77</td>
<td>102 ± 1</td>
</tr>
<tr>
<td>0.500</td>
<td>0.509 ± 0.003</td>
<td>0.52</td>
<td>102 ± 1</td>
<td>0.509 ± 0.003</td>
<td>0.54</td>
<td>102 ± 1</td>
</tr>
<tr>
<td>Zn (II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.100</td>
<td>0.110 ± 0.004</td>
<td>3.36</td>
<td>110 ± 4</td>
<td>0.110 ± 0.004</td>
<td>3.36</td>
<td>110 ± 4</td>
</tr>
<tr>
<td>0.300</td>
<td>0.303 ± 0.008</td>
<td>2.74</td>
<td>102 ± 3</td>
<td>0.303 ± 0.008</td>
<td>2.74</td>
<td>102 ± 3</td>
</tr>
<tr>
<td>0.500</td>
<td>0.484 ± 0.009</td>
<td>1.94</td>
<td>97 ± 2</td>
<td>0.484 ± 0.009</td>
<td>1.94</td>
<td>97 ± 2</td>
</tr>
</tbody>
</table>

All the experiments were run in triplicates, indicated with mean ± standard deviation.

Table 4. Interday determination of Cu$^{2+}$, Ni$^{2+}$ and Zn$^{2+}$ in spiked tap water.

<table>
<thead>
<tr>
<th>Ion Species Spiked (ppm)</th>
<th>Found (ppm)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
<th>Found (ppm)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu (II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.100</td>
<td>0.11 ± 0.01</td>
<td>9.31</td>
<td>107 ± 10</td>
<td>0.11 ± 0.01</td>
<td>10.30</td>
<td>112 ± 12</td>
</tr>
<tr>
<td>0.300</td>
<td>0.306 ± 0.007</td>
<td>2.41</td>
<td>102 ± 2</td>
<td>0.31 ± 0.01</td>
<td>3.28</td>
<td>103 ± 3</td>
</tr>
<tr>
<td>0.500</td>
<td>0.517 ± 0.007</td>
<td>1.38</td>
<td>103 ± 1</td>
<td>0.519 ± 0.009</td>
<td>1.70</td>
<td>104 ± 2</td>
</tr>
<tr>
<td>Ni (II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.100</td>
<td>0.102 ± 0.006</td>
<td>5.97</td>
<td>102 ± 6</td>
<td>0.098 ± 0.007</td>
<td>7.56</td>
<td>98 ± 7</td>
</tr>
<tr>
<td>0.300</td>
<td>0.303 ± 0.006</td>
<td>2.05</td>
<td>101 ± 2</td>
<td>0.300 ± 0.008</td>
<td>2.62</td>
<td>100 ± 3</td>
</tr>
<tr>
<td>0.500</td>
<td>0.503 ± 0.006</td>
<td>1.24</td>
<td>100 ± 1</td>
<td>0.500 ± 0.006</td>
<td>1.26</td>
<td>100 ± 1</td>
</tr>
<tr>
<td>Zn (II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.100</td>
<td>0.106 ± 0.007</td>
<td>6.70</td>
<td>106 ± 7</td>
<td>0.104 ± 0.006</td>
<td>6.27</td>
<td>104 ± 6</td>
</tr>
<tr>
<td>0.300</td>
<td>0.30 ± 0.01</td>
<td>3.14</td>
<td>102 ± 3</td>
<td>0.31 ± 0.01</td>
<td>3.34</td>
<td>103 ± 3</td>
</tr>
<tr>
<td>0.500</td>
<td>0.49 ± 0.01</td>
<td>2.98</td>
<td>97 ± 2</td>
<td>0.49 ± 0.01</td>
<td>3.14</td>
<td>98 ± 3</td>
</tr>
</tbody>
</table>

All the experiments were run in triplicates, indicated with mean ± standard deviation.

No significant difference was observed for both methods. From Table 2, it can be observed that the $\gamma^{-1}$ value is small for the three compounds in and this suggests that they are good methods for multivariate determination. The LOD of Zn$^{2+}$ is observed to be relatively higher than others. This suggests that there is a relatively higher spectral overlap and causing defection of the net analytical signal value, which may due to the tolerability of Zn model in presence of interference.

Application on Spiked Samples

It is sometimes argued that the real predictive value of any calibration models cannot be judged solely by using synthetic mixtures. It has to be tested on real samples that are not included in the previous steps. For this effect, tap water samples spiked with known amount of metals were analyzed in order to evaluate the precision, accuracy and recovery of the suggested method. The results for intraday and interday validation of ternary mixture are shown in Table 3 and Table 4. As can be seen, the prediction ability of both models for all the analytes in a real matrix is in good agreement.

Influence of pH

Once a satisfactory model has been developed, it would be good if it remains “robust” to small variation in the experimental conditions. The effect of pH on the absorption spectra of metal complexes was studied over the pH range of 3-12 in the presence of Triton X-100 adjusted by diluted HNO$_3$ and H$_2$SO$_4$. The pH effect was carried out by adjusting the pH value of the spiked samples using diluted HNO$_3$ and H$_2$SO$_4$.
Table 5. Tolerance ratio for foreign ions in the determination of 0.30 ppm of Cu\(^{2+}\), Ni\(^{2+}\) and Zn\(^{2+}\) mixture.

<table>
<thead>
<tr>
<th>Tolerated ratio</th>
<th>Cu</th>
<th>Ni</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 100</td>
<td>Cd(^{2+}), Mg(^{2+}), Mn(^{2+})</td>
<td>Al(^{3+}), As(^{5+}), Ca(^{2+}), Cd(^{2+}), Cr(^{3+}), Mg(^{2+}), Mn(^{2+}), Se(^{4+})</td>
<td>Fe(^{3+})</td>
</tr>
<tr>
<td>&gt; 20</td>
<td>Al(^{3+}), As(^{5+}), Ca(^{2+}), Cr(^{3+}), Se(^{4+})</td>
<td>Mg(^{2+})</td>
<td></td>
</tr>
<tr>
<td>&gt; 1</td>
<td>Fe(^{3+}), Hg(^{2+}), Pb(^{2+})</td>
<td>Hg(^{2+}), Pb(^{2+})</td>
<td>Al(^{3+}), Ca(^{2+}), Fe(^{3+}), Hg(^{2+}), Pb(^{2+}), Mn(^{2+}), Se(^{4+})</td>
</tr>
</tbody>
</table>

NaOH solutions. It was observed that the absorbance of the complexes were increased by increasing pH from 3.3 to 5.9 and remained constant between 5.9 and 7.1 (where the inaccuracy were less than 2%). Although the absorbance kept increasing as pH went up, the inaccuracy became higher (more than 5%) and the spectrums lost their characteristics after pH 8.4.

**Influence of Ammonium Acetate**
Ammonium acetate is a relatively cheap chemical that provide colourless solution used for controlling of final solution around pH 6.7. The effect of ammonium acetate concentration was studied as well. As expected, the result indicates there are no significant differences while applying from 0.25 mL to 5.00 mL of 1.00 mol dm\(^{-3}\) of ammonium acetate.

**Influence of Surfactants**
When the concentration of surfactant exceeds the critical micelle concentration (CMC), micelles are formed homogeneously in aqueous solution. The non-polar core of micellar system provides high solubilization capacity for TAN and its complexes. This allows development of spectrophotometry procedures with enhanced absorbance of TAN-metal complexes, thereby avoiding the solvent extraction. Furthermore, it also act as nonionic detergent that ease the cleaning process. In this work, the function of Triton X-100 as a non-ionic surfactant in the colour reactions was studied and the optimum working range of the surfactant has been investigated. The results show that 0.1 - 0.2% of Triton X-100 (v/v) was insufficient to fully dissolve the excess TAN and its complexes which causing error in estimation, although the concentration of Triton X-100 is greater than the CMC (0.22 - 0.24 mM); whereas there were no significant differences in the spectra and predicted values while concentration of Triton X-100 was increased from 0.4 to 2.0%. Consequently, the concentration of Triton X-100 is maintained at 0.4% as to obtain the optimum performance.

Besides that, Triton X-100 was substituted with 50%-methanol as well as Tween-20. Fluctuated and comparatively low absorbance spectra were obtained in 50%-methanolic solution. This is due to the volatility of methanol and the solubility of TAN and its complexes. Conversely, the spectra acquired using Tween-20 and Triton X-100 are similar, as the predicted values were in acceptable range (variation less than 5%).

**Influence of Foreign Ions**
The influence of some possible foreign species was also investigated by analyzing a standard mixture solution contains 0.300 ppm of each Cu\(^{2+}\), Ni\(^{2+}\), Zn\(^{2+}\), and with which increasing amounts of interfering species were added. The tolerable limit of a foreign species was taken as the inaccuracy that not greater than 5% and are depicted in Table 5. The experimental result showed that Hg\(^{2+}\), Pb\(^{2+}\) and Fe\(^{3+}\) cause cationic interferences as a result of formation of coloured complexes with TAN which absorb within study wavelengths. If Zn\(^{2+}\) is to be simultaneous determined, Cd\(^{2+}\), Cr\(^{3+}\), Mg\(^{2+}\) ions must be absent because they produce interferences even at concentration levels similar to the analyte concentration.

**Stability of Samples**
The stability of complexes was investigated by
comparing the absorption spectrum and the estimated concentrations of the solutions at different times of interval. The samples containing ternary mixture of Cu(II), Ni(II) and Zn(II) in water samples is made feasible by multivariate calibration. This technique consists of a simple, rapid and inexpensive procedure, which does not require tedious pretreatment. Good agreement in prediction ability has been clearly demonstrated. Analysis of the results for the three-component system revealed that there are no significant differences in the ability of prediction between the PLS-2 and PCR procedures for zero order data.

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