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Novel Manganese Colorimetric Chemosensing Investigations of *Indigofera macrophylla* Schum (Thonn.) Stem and Leaf dye Extracts

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Purpose: To determine the chemosensor ability of dye extracts obtained from the stem and leaves of *Indigofera macrophylla* Schum (Thonn.), a scandent or climbing shrub belonging to the family Fabaceae.

Method: The dye extracts were obtained from the stem and leaves of the plant after optimizing the method of extraction, the concentration of base for precipitation as well as the contact time between base and aqueous plant extracts. The dyes were investigated for their colorimetric chemosensor properties using procedures such as screening of metals and non-metals, selection of analytical wavelengths, optimization of solvents, reaction temperature and time. Validation was carried out for the determination of manganese from aqueous solution by preparation of calibration curve, accuracy and reproducibility. Interference liabilities of the new method for manganese were studied in the presence of common cation and anions.

Results: The dyes were obtained in good yield and gave pink colours. Spot tests revealed the selective detection of manganese from the two dye extracts. These two dyes; the stems (DS and leaves (DL) dyes of *Indigofera macrophylla*) proved to be excellent colorimetric chemosensors for manganese in an aqueous solution. Linearity in Beer's law plot were obtained within the concentration range of 10-50 µg/mL (r^2 = 0.996 and 0.999 for DS and DL respectively). Accuracy gave relative errors generally less than 2.0% while for the precision, the relative standard deviations were less than 0.8%. There was no interference with common cations such as Cr^{3+}, Cu^{2+}, Zn^{2+} and anions such as CN^- and NO_2^-

Conclusion: The natural dyes obtained from *Indigofera macrophylla* could be excellent sources of chemical principles for the detection and determination of manganese in aqueous solutions.

Keywords: *Indigofera macrophylla*, Natural dyes, Manganese, Colorimetric chemosensor, Interference studies

INTRODUCTION

A chemosensor can be regarded as ''a receptor that interacts with an analyte producing a detectable change in a signal'' (Anslyn, 2007). Colorimetric chemosensors are designed in such a way that the receptor and signaling units are either fused together into one unit or connected by some unsaturated groups (Li *et al*, 2011). The real advantage of colorimetric chemosensor is that the recognition event is visible to the naked eye with change in color and the detection can be real time and on a molecular scale (Czarnik, 1992; Spichiger-Keller, 1998).

Colorimetric methods can conveniently and easily monitor target ions in the visible range with high sensitivity, specificity, simplicity, low cost, and rapid tracking of analytes in biological, toxicological, and environmental samples (Li *et al*, 2011).

Manganese is an element essential to the proper functioning of both humans and animals, as it is required for the functioning of many cellular enzymes e.g. manganese superoxide dismutase, pyruvate carboxylase and can serve to activate many others e.g. kinases, decarboxylases, transferases, hydrolases (IPCS,2002). Manganese can exist in

eleven (11) oxidative states; the most environmentally and biologically important manganese compounds are those that contain Mn^{2+}, Mn^{4+} or Mn^{7+} (USEPA, 1994).

However, elevated manganese levels can cause human neurotoxicity. Notably, workers exposed to high airborne manganese levels are at elevated risk of developing parkinsonism (Cersosimo and Koller, 2006), and adverse effects of exposure to elevated Manganese in drinking water have been observed in children (Khan *et al*, 2011). These risks can be lowered once chemosensing methods for manganese are implemented. There is little research in the colorimetric chemosensing determination of manganese (Kim *et al*, 2014, Lee *et al*, 2014, Narayanan and Han, 2017) and none on using natural dyes to sense manganese.

Natural dyes generally pose little or no risk to human health therefore they are preferred to synthetic dyes. The primary aim of this research work is to use natural dyes obtained from the stem (DS) and leaf (DL) of *Indigofera macrophylla* Schum (Thonn.) to detect the presence of manganese upon chelation in aqueous solutions. Sensitive and selective colorimetric methods for the determination of manganese in aqueous solutions were thereafter developed.

EXPERIMENTAL

Chemical and reagents

Acetone, Ethanol, Ethyl Acetate, Hexane, Methanol (All the solvents were obtained from Sigma-Aldrich), Potassium permanganate ($KMnO_4$), Copper sulphate ($CuSO_4$), Zinc Chloride ($ZnCl_2$), potassium dichromate ($K_2Cr_2O_7$), Sodium nitrite ($NaNO_2$) and Sodium cyanide (NaCN) (All these salts were obtained from BDH chemical limited, Poole, England).

Natural dyes utilized

The dyes used were extracted from the stem (DS) and leaf (DL) of *Indigofera macrophylla* Schum (Thonn.). The dyes were utilized after separation from the mother liquor and freeze dried.

Instrumentation

Thomas-Willey milling machine, Mettler analytical balance (Ohaus, USA), LTE LYOTRAP PLUS Freeze dryer (LTE Scientific Limited, Great Britain), UV-VIS spectrophotometer equipped with 1 cm quartz cells (Spectrumlab 752s), Scanning UV-VIS spectrophotometer equipped with 1cm quartz cells and Thermostated water Bath (Uniscope).

Plant Material

Fresh *Indigofera macrophylla* leaves and stems were collected from Ido Local Government area along Eruwa road, Oyo state. Flowering sample of the plant was taken to Forestry Research Institute of Nigeria (FRIN) for identification and authentication. It was identified by Mr. Oba and it was authenticated (FHI number - 110327) by Mr. Adeniji, a staff at the herbarium in FRIN to be *Indigofera macrophylla.*

Extraction of the Dyes

The stem and leaves were collected and dried. Thereafter, the plant parts were grinded using the Thomas-Walley milling machine and transferred into an aspirator bottle. Water was used for the extraction of the plant. This was left for three days (72 hours) for proper cold extraction. The dye was precipitated out of the crude aqueous extract using 2 M NaOH. The precipitated dye obtained was decanted off the solvent of extraction and concentrated down to a very small volume (close to dryness) on a water bath. The dye extracts obtained were then freeze-dried.

Preliminary phytochemical screening

The dye extracts were investigated for their phytochemical contents. Tests for tannins, glycosides, sterols, terpenoids, flavonoids, alkaloids, phenols and saponins were conducted using standard test guidelines as reported by Edeoga *et al*, 2005.

Preparation of stock solutions

A 10 mg quantity of potassium permanganate (source of manganese) was dissolved into 10 mL of distilled water to give a 1 mg/mL stock solution. For the dye solutions, 10 mg of DS and DL were separately dissolved into 10 mL of distilled water to give a 1 mg/mL stock solution.

Measurement of UV-VIS Absorption spectrum of sample solutions

A 0.5 mL aliquot of the manganese solution was diluted with acetone to 5 mL. The absorbance of the manganese solution was then recorded from 190 – 900 nm to determine its maximum wavelength. Similarly, a 0.5 mL aliquot of each of the dyes (DS and DL) was diluted with acetone to 5 mL individually and the UV-VIS absorption spectrum was recorded for each sample. For the dye-Mn adducts, a 0.5 mL aliquot of the manganese solution was pipetted into a test-tube and 0.5 mL aliquot of the dye's stock solution was added and mixed for 10 seconds. The immediate pink coloured complex formed was allowed to stay at room temperature for 10 minutes. The absorption spectra of the DS- Mn Adduct and DL-Mn Adduct were recorded from 190 – 900 nm (Adegoke *et al*, 2004). In all instances,

acetone was used as the blank solvent for the background correction on the spectrophotometer.

Optimization studies

Optimization of diluting solvents

Four solvents were used for the optimization. The solvents used were ethanol, methanol, water and acetone. This was done in duplicates. A quantity (0.5 mL) of 1000 µg/mL of the dye was added to 0.5mL of 1000 µg/mL of manganese. The reaction was maintained at 30 ºC for 10 minutes. The reaction vessel was immersed in ice-cold water bath and then the volume diluted to 5 mL with acetone. The absorbance was measured using a UV/Vis spectrophotometer at the optimal wavelength of absorption. The reaction was repeated with dilution with ethanol, methanol and water in each case.

Optimization of Temperature

The method of the steepest ascent was adapted for this assessment (Karnes and March, 1993). This was done at four different temperatures. The temperatures were 30 ºC, 50 ºC, 60 ºC and 70 ºC. The procedure was done in duplicates. 0.5 mL of 1000 µg/mL of the dye was added to 0.5 mL of 1000 µg/mL of manganese. The reaction took place at 30 ºC for 5 and 20 minutes and finally diluted with the optimal diluting solvent up to 5 mL final volume. The procedure was repeated in different test-tubes at 50 ºC, 60 ºC and 70 ºC. The reaction tubes were cooled to room temperature in ice-cold bath and the volume diluted to 5 mL final volume. The absorbance of each solution was measured using a UV/VIS spectrophotometer at the optimal wavelength of 520 nm to obtain the optimum temperature for the coupling reaction.

Optimization of Time

Seven reaction times were tested for the optimization of time for the dye-metal adduct. The reaction times were 0, 2, 5, 10, 20, 25 and 30 minutes respectively at the optimal temperature of 30 ºC. This was done in duplicates.

A 0.5 mL aliquot of 1000 µg/mL of the dye was added to 0.5 mL of 1000 µg/mL of manganese. The reaction took place at the selected optimal temperature for 0, 2, 5, 10, 20, 25 and 30 minutes. After each period of time, the reaction was terminated by making the solution up to 5mL with the optimal diluting solvent. The absorbance was measured using a UV/VIS spectrophotometer at the optimal wavelength of absorption at 520 nm.

Stoichiometric ratio determination

Job's method of continuous variation was adopted for this study (Adegoke *et al*, 2014). A 1 mg/mL quantity of both the dyes and the manganese solutions were prepared. Into ten different test-tubes 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 mL of Manganese was pipetted. Different aliquots of the dye solutions were added to the each of the test-tubes to make up the volume to 1mL i.e. 1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 and 0 mL respectively. The mixtures were incubated at the optimum temperature and time (30 ºC, 25 mins for DS and 30 ºC, 5 mins for DL) and made up to 10mL with the optimum diluting solvent.

Validation Studies

Calibrations for the determination of manganese were carried out using the optimal analytical conditions as described above. In each instance, 0.5 mL of the 1 mg/mL stock of DS and DL were used for the respective concentrations of manganese solutions. The tubes for DS were incubated at 30 ºC for 25 mins while that for DL were incubated at 30 ºC for 5 mins. At the end of each reaction time, contents of sample tubes were diluted to 5 mL with solvents. Linear regression analysis was used to calculate the slope, intercept and the correlation coefficient (r) of each calibration line. The calibration curve was prepared on each of three successive days using duplicate samples for each analyte concentration and the average pooled data was adopted.

The limits of detection and quantitation (LOD and LOQ) were calculated according to the current ICH guidelines as 3.3 and 10 standard deviation of the blank divided by the slope of the calibration curve respectively (ICH, 1995). Accuracy and repeatability of the new methods were carried out on three successive days using concentration levels of 12.5, 27 and 42 µg/mL of aqueous manganese solution. For each concentration level, 6 replicate sample determinations were carried out for each day.

Interference liabilities studies

The preparations of sample solutions were repeated with each of the dyes but using each source of common metals ($CuSO_4$, $ZnCl_2$ and $K_2Cr_2O_7$) and anions ($NaNO_2$ and NaCN) as reactants. The solution was allowed to stand at 30 ºC for 10 min and finally diluted with water up to 5 mL final volume after cooling in ice-bath. The absorption spectra for each solution were recorded within the wavelength range of 190 – 900 nm.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of tannins, glycosides, steroids, terpenoids, flavonoids, alkaloids and saponins in the stem and leaf dye extracts (DS and DL) of *Indigofera macrophylla* out of the eight phytochemicals that were tested for. Phenol was absent in the two dye extracts. Tannins were found to be present in very large quantities giving rise to highly intense brownish green coloration.

Formation of the dye-manganese adducts

The reaction showed that the addition of the DS and DL to manganese created a pink coloured product at room temperature. The purple colour of the manganese changed to pink upon the addition of the dyes. This indicates the formation of a new compound. This shows that manganese can be sensed

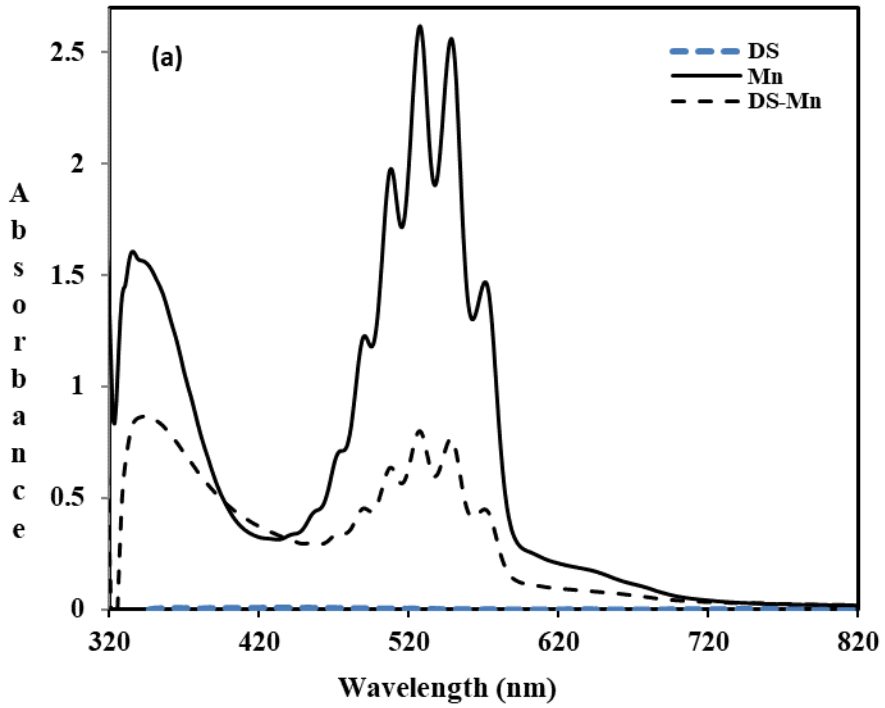
Selection of the analytical wavelength

The main aim of this experiment was to generate a coloured adduct upon the reaction of manganese and the dyes, that would have a significant absorption in the visible region where there was little or no interference from irrelevant absorptions. Therefore, 520 nm was selected as the working wavelength.

The selection of analytical wavelength was achieved by determining the wavelength with a distinct difference in absorptivity between the dyes, manganese and the dye-manganese adducts. Fig. 1a and b are the overlaid absorption spectra showing the difference in absorbance of the dyes, manganese and the dye-Manganese adduct for the scanning range of 320 - 820 nm. At 520 nm, the chemosensor product showed a hypochromic shift. However, in this study, this was adequate to permit the determination of manganese at the visible region.

Electronic absorption spectra

The overlaid absorption spectrum of the dyes, manganese and the dye-manganese adducts are shown in figs 1a and 1b for dyes DS and DL respectively. The three show significant absorptions in the visible region. The major absorptions of manganese were around 430 nm, 470 nm, 520 nm and 600 nm, while the prominent absorption in DS and DL were at 430 nm. In the DS-Manganese adduct formed, a hyperchromic shift was observed at 430 nm, 470 nm and 490 nm, while a hypochromic shift was observed at 520 nm and 600 nm. In the DL-Manganese adduct formed, a hyperchromic shift was observed at 430 nm and 470 nm, while a hypochromic shift was observed at 490 nm, 520 nm and 600 nm.



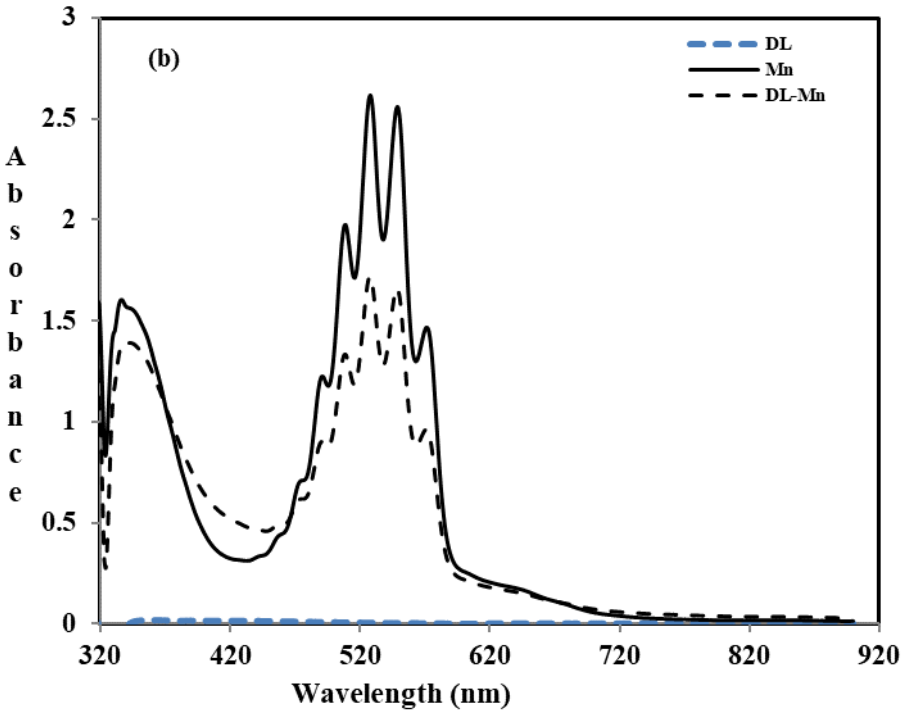


Fig. 1: Electronic absorption spectra of the dyes, manganese and the dye-manganese adducts [(a) - stem dye and (b) - leaf dye]

Optimization of the reaction conditions

The reaction conditions for the colorimetric determination of manganese in solution were optimized. The reaction conditions optimized were principally diluting solvent, temperature and time.

Four solvents were used for the optimization of diluting solvent. The solvents used were ethanol, methanol, water and acetone. The absorbance was recorded in duplicates at 520 nm. The results for these solvents' optimization studies are presented in fig. 2. In both instances while using the leaf and stem dyes, water gave the optimum absorbance compared to polar solvents ethanol and methanol and the semi-polar solvent, acetone.

The method of steepest ascent (Karnes and March, 1993) was used to investigate optimum temperature that would favour the formation of the desired product. In this investigation, four different temperatures at two levels of reaction times were studied. The temperature levels used were 30 ºC, 50 ºC, 60 ºC and 70 ºC each at 5 and 20 minutes. This was carried out at the selected wavelength of 520 nm. The results obtained during the optimization process are presented in fig. 3.

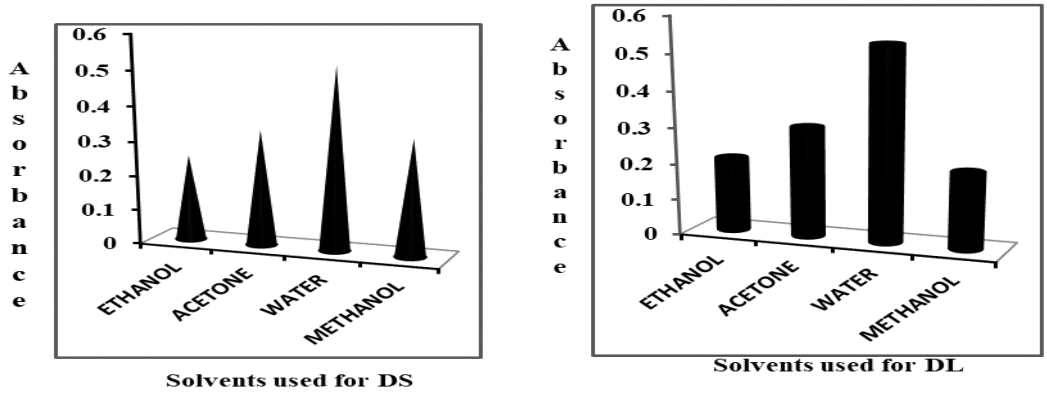


Fig. 2: Optimization of solvent for DA and DB respectively for the Colorimetric chemosensor of Manganese

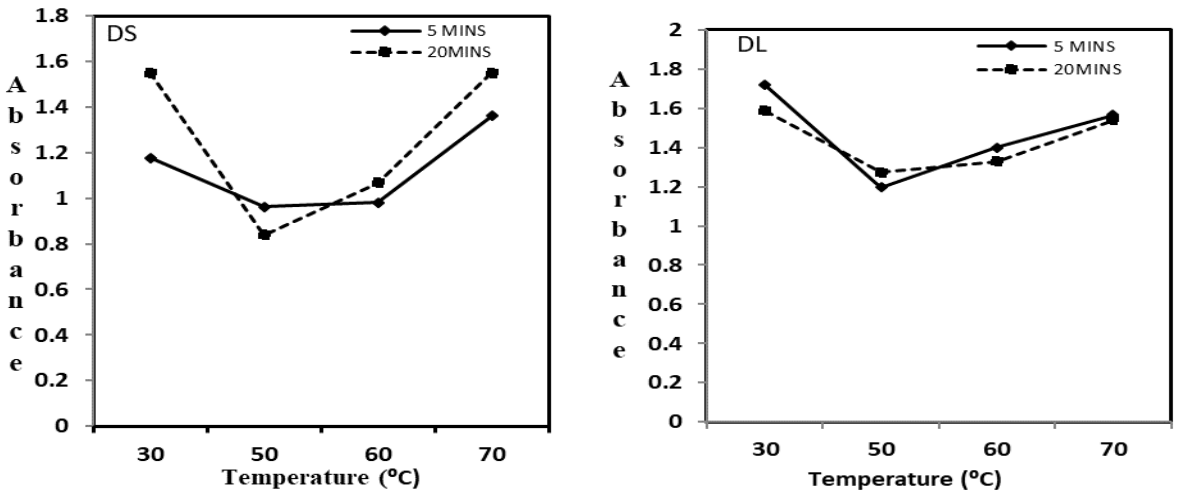


Figure 3: Optimization of reaction temperature between manganese and *Indigofera* plant dye extracts

The optimal temperature selected was 30 ºC for both DS and DL with absorbance values of 1.733 and 1.178 at 5 minutes for DS and DL respectively and 1.587 and 1.550 at 20 minutes for DS and DL, respectively. There was an elevation in absorbance at 70 ºC but this was most likely due to the degradation of Manganese at 70 ºC. Therefore 30 ºC was used as the most suitable temperature for subsequent analyses in the optimization of time and other procedures. Potassium permanganate is highly sensitive to change in temperatures, that is, the higher the temperature the more erratic the reaction is. The valleys observed in the optimization of temperature can be justified by the erratic behavior of potassium permanganate at high temperatures. Usually high temperatures lead to catalytic decomposition of Mn^{7+} to lower oxidation states.

For the time optimization reactions, analysis was carried out at 30 ºC using 0, 2, 5, 10, 20, 25 and 30 minutes respectively. This was done in duplicates. The results are presented in Fig. 4. The suitable reaction time for DS was 25 minutes with absorbance value of 2.006 and 2.009 respectively while for DL it was 5 minutes with absorbance value of 1.427 and 1.426, respectively.

From the results obtained as presented in fig. 4, it can be observed that DL posed an erratic behavior on its reaction with manganese which could be backed up by the fact that after 5-10 minutes of the reaction time, the color of the adduct kept fluctuating between pink, pinkish-wine and wine, which influenced the haphazard change in the absorbance value over time. Thus, the most suitable reaction time was 5 minutes and this was selected for subsequent investigations.

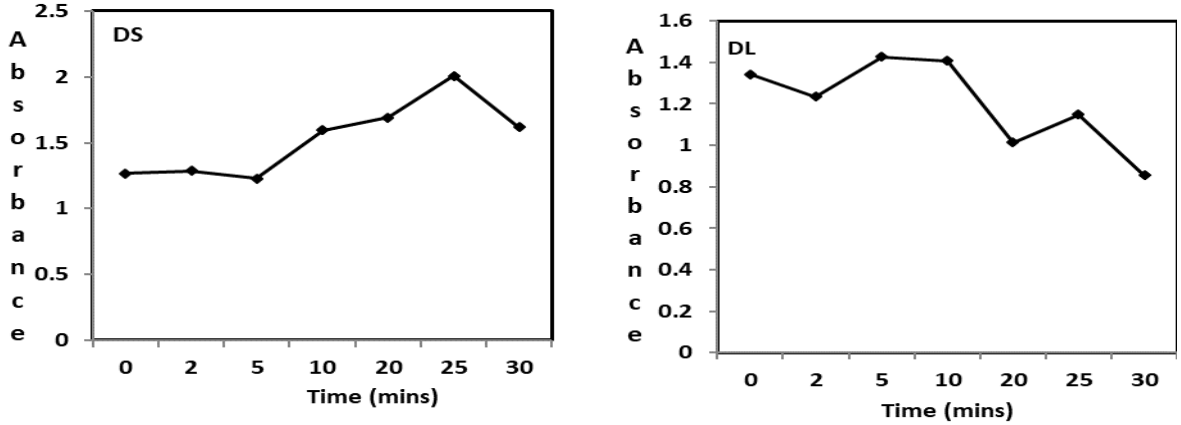


Fig. 4: Effects of reaction time for the reaction between manganese and *Indigofera* plant extracts

Stoichiometric ratio determination

The stoichiometry of the reaction between the dyes and manganese was investigated by Job's method of continuous variation. The absorbances of the new adduct formed were found to vary with the stoichiometric ratio of the dyes and manganese. The optimal mole ratio which was 0.3:0.7 of dye: manganese was used for subsequent determinations. The results are presented in fig. 5. Since the dye extracts contain several bioactive principles, any of such components can be involved in the reduction of Mn^{7+} to lower oxidation states to give the pink colour observed. The uneven stoichiometry is anticipated as the dye is not a classically distinct or known chemical moiety. It is possible that more than one secondary plant metabolites may be involved in the reductive process. The likelihood of a single metabolite with several reactive sites cannot also be ruled out.

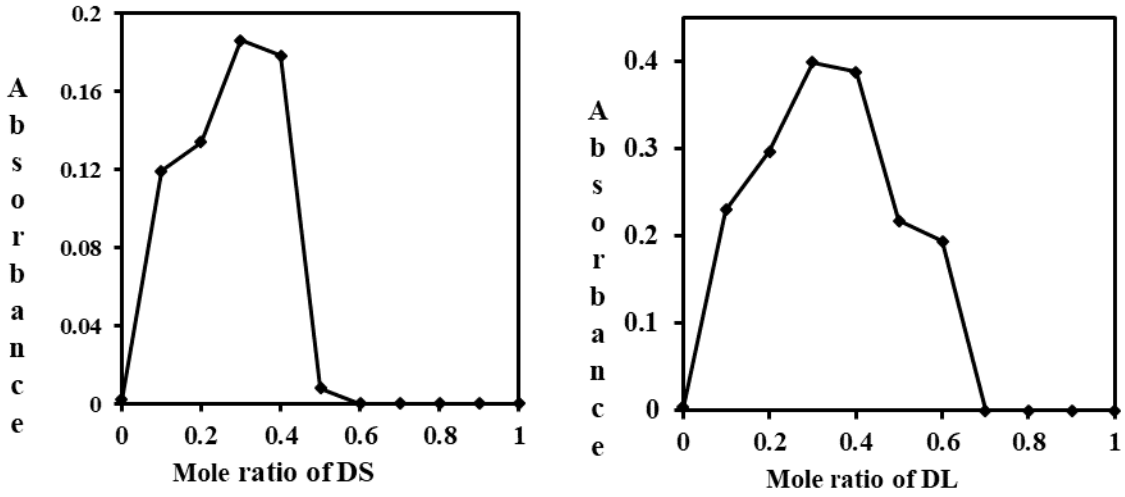


Figure 5: Stoichiometric ratio determination for the reaction between the dyes and Manganese

Validation of the new chemosensor methods for manganese determination

The linearity of response of the new colorimetric chemosensor method was determined. It was observed that the dyes gave a linear absorbance value corresponding to manganese concentration within the range 5-50 µg/mL. The calibration curve of the new colorimetric chemosensor method was prepared under the optimized conditions by the absorbance as a function of corresponding concentration on each of the three consecutive days.

The basic analytical parameters for the calibration curves produced for the new chemosensor method for Mn using DS and DL are presented in Table 1. A positive correlation of absorbance was observed along with a coefficient of determination (r^2) of 0.9960 for DS and 0.999 for DL. The LOD which is defined as the lowest amount of an analyte in a sample which can be detected but not necessarily quantitated was 0.0332 µg/mL for DS-Mn adduct and 0.3431 µg/mL for DL-Mn adduct. The LOQ which is the smallest amount that can be quantitatively determined is 1.00671 µg/mL for DS-Mn adduct and 1.0397 µg/mL for DL-Mn adduct. The LOD and LOQ of DS-Mn adduct is lower compared to the LOD and LOQ of DL-Mn adduct which simply means DS could be regarded as the better dye for the detection and quantitation of manganese in aqueous solution. Thus by this chemosensor technology, concentrations as low as 0.03 and 0.34 ppm Mn can be detected by DS and DL, respectively.

The results of the inter-day assessment of the accuracy and precision for the DS-Mn and DL-Mn adducts are presented in Table 2 while that of the intra-day assessment are presented in Table 3. For the inter-day assessment, the accuracies were generally of the order of 99.99 – 101.63% with precision as low as 0.18–0.68% (RSD). The close percent recovery, the low errors and relative errors obtained for DS and DL shows good accuracy while the low standard deviations and relative standard deviation shows acceptable precision. From the obtained results it can be deduced that the percentage recovery, the standard deviations, relative standard deviations, errors and relative errors showed that these parameters passed the required criteria recommended by ICH. The values obtained for the intra-day assessment of accuracy and repeatability also points to the suitability of this new method for the assay of Mn as the relative errors were all less than 2%. One clear-cut observation from the results obtained is that

DS appears a better chemosensor than DL as the assessments of accuracy and precision gave errors and relative standard deviations that were profoundly lower. This result seems to stem from the absorption spectra obtained (Fig. 1a and 1b) where a pronounced hypochromic shift was observed when DS was used as opposed to the marginal shift observed for DL.

Table 1: Analytical and Validation parameters for the colorimetric determination Manganese ion

Parameter	DS-Mn	DL-Mn
Beer's law limits (µg mL^{-1})	5-50	5-50
Limit of detection LOD (µg mL^{-1})	0.0332	0.3431
Limit of quantitation LOQ (µg mL^{-1})	1.00671	1.0397
Regression equations*		
Intercept, c	0.0109	0.0275
Standard deviation of intercept	0.00078	0.01181
Slope, m	0.0149	0.0136
Standard deviation of slope	0.00062	0.00117
95% C. I. of slope	0.00155	0.00291
95% C. I. of intercept	0.00699	0.02934
Correlation coefficient, r	0.996	0.999

* y= mx + c, where y is the absorbance for concentration x in µg mL^{-1};
C.I. = confidence interval

Table 2: Inter-day accuracy and precision for the determination of Mn by the DS and DL

Dye-Mn	Amount taken (µg/mL)	Amount found (µg/mL)	Recovery (%)*	S.D*	RSD (%)	Error	Relative error (%)
DS-Mn	12.5	12.503	99.99	0.085	0.68	0.03	0.24
	27.0	27.1	100.36	0.081	0.30	0.10	0.36
	42.0	42	100.00	0.077	0.18	0.08	0.19
DL-Mn	12.5	12.7	101.59	0.085	0.68	0.20	1.63
	27.0	27.44	101.63	0.084	0.31	0.44	1.64
	42.0	42.14	100.32	0.085	0.21	0.14	0.33

*n=12

Interference liabilities studies

Achieving high selectivity for the analyte of interest (manganese) over a complex background of potentially competing species is a challenge in evaluating chemosensors. Thus interactions between the dyes (DS and DL) and other common metals and anions were investigated by UV–Vis spectroscopy.

The preparations of sample solutions were repeated with each dye sample but using each of common metals ($CuSO_4$, $ZnCl_2$ and $K_2Cr_2O_7$) and anions ($NaNO_2$ and NaCN) as reactants. The resultant solutions were allowed to stand at room temperature of 30 ºC for 10 min and finally diluted with water up to 5 mL final volume and the absorbance at 190 – 900 nm was recorded. The absorption spectra of the dyes in the presence of several other metals and anions are presented in fig. 6.

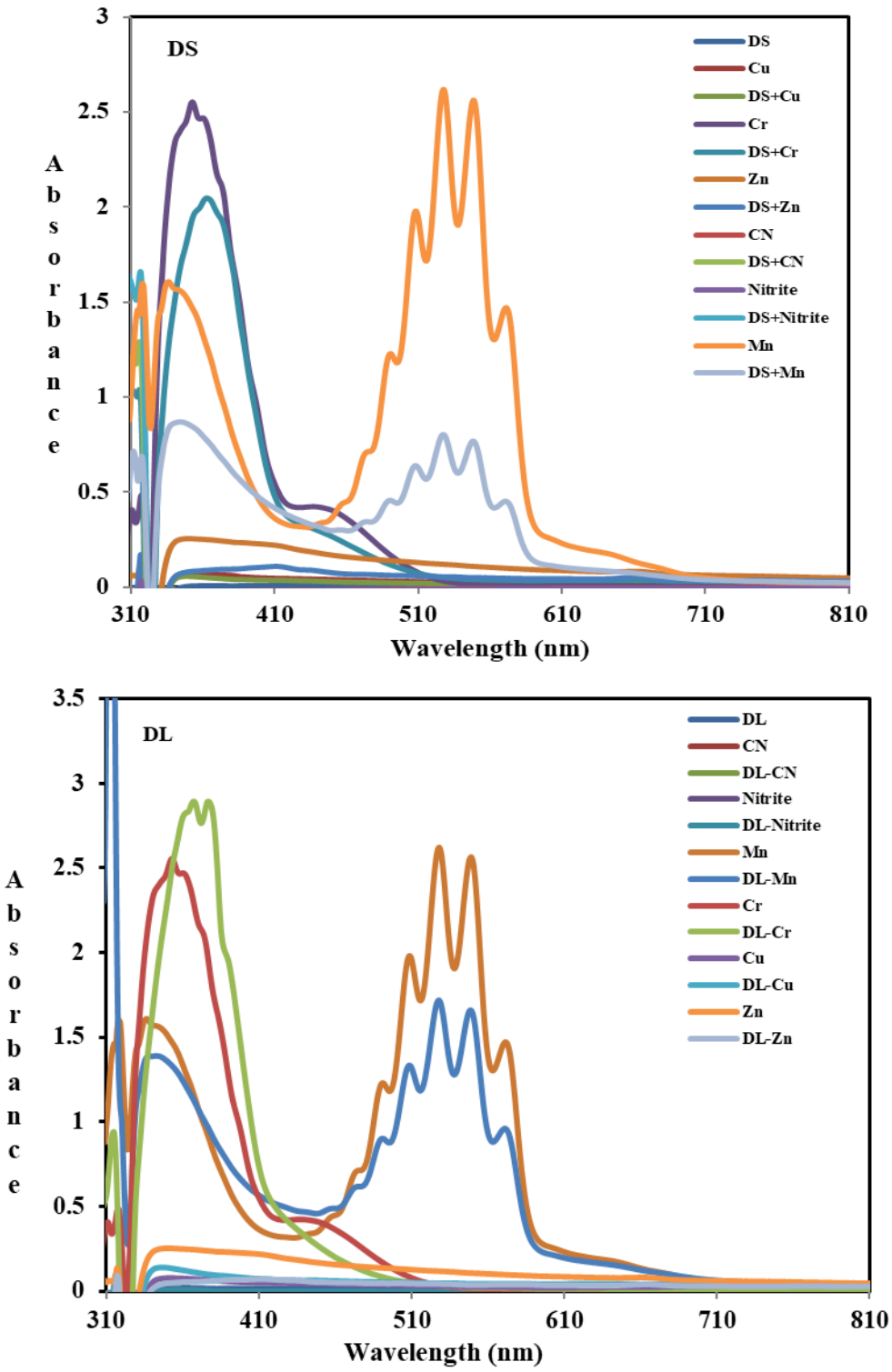


Fig. 6: Interference liabilities with other common cations and anions

Table 3: Intra-day accuracy and repeatability for the assessment of Mn in aqueous solution

Dye-Mn	Amount taken (µg/mL)	Amount found (µg/mL)	Recovery (%)*	S.D*	(R.S.D) (%)	Error	Relative error (%)
DS-Mn							
	12.5	12.51	100.04	0.110	0.88	0.01	0.08
	27.0	27.02	100.07	0.087	0.32	0.02	0.07
	42.0	41.97	99.93	0.063	0.15	0.03	0.07
DL-Mn	12.5	12.72	101.74	0.094	0.75	0.22	1.76
	27.0	27.47	101.72	0.094	0.35	0.47	1.74
	42.0	42.13	100.31	0.094	0.23	0.13	0.31

*n=4

It was observed that Cu^{2+}, Zn^{2+}, NO_2^- and CN^- did not produce any colour change upon reaction with both DS and DL and there was no significant absorption in the visible region where manganese produced noticeable colours with both DS and DL dyes. It was actually easy to distinguish the reaction between the dyes and Mn due to the prominent absorption in the visible region compared to the other anions and cations. The pink colour change and the absorption at 520 nm were not observed when the dyes reacted with other metals and anions. Thus, this has helped in selectively distinguishing Manganese from all other anions and cations and thus establishing the dyes from *Indigofera macrophylla* are selective and sensitive colorimetric chemosensor.

The Cr^{3+} sample gave a mild colour change from orange to a faint orange or yellow colour upon reaction with both dyes. A slight absorption in the visible region around 425 nm was observed upon reaction with DS and 410 nm upon reaction with DL. This absorption was not prominent and can be easily differentiated from the prominent absorption of manganese in the visible region of the spectrum which was around 520 nm for both dyes. It also implies that the dyes, DS and DL, may be able to serve as suitable chemosensors for Cr^{3+} alongside Mn^{7+} without undue interference in terms of both colour derivatives produced and absorption maxima for determination of the respective cations.

Possible mechanism of chemosensing action of Indigofera dyes

The lack of interference by common anions studied provides the explanation that a reductive mechanism propelled by the dyes on the metal ions appears prominent. Since naturally occurring products contain myriads of secondary plant metabolites, such reactions are provided by any of such components. In this particular case, a high content of tannins was found from the preliminary phytochemical screening. Since tannins are complex polyphenolic compounds (Porter, 1992), these may have interacted with the manganese to give rise to drastic reduction in both the colour of the manganese solution and the intensity of the peak at 520 nm. The presence of flavonoids may have contributed additional reductive process or may provide complexing ability for the manganese cation.

CONCLUSION

Manganese was successfully detected from aqueous solution using the dye extracts obtained from the stem and leaves of *Indigofera macrophylla*. The results obtained showed that the dye extracts sensitively and selectively detected manganese without undue interference from other common cations and anions, especially zinc, copper, chromium, cyanide and nitrite. As a result of this fact, a novel, cheap, effective, safe and simple method for colorimetric detection of Mn was discovered. A mechanism whereby any of the components of the dye extract is reducing manganese is suspected. Further work will focus on the isolation of the particular component for possible full elucidation of the mechanism of chemosensor action. These dyes are safer to handle and utilize compared to synthetic dyes which could pose some hazard to human health and the environment.

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