SPECTROPHOTOMETRY MOLE RATIO AND CONTINUOUS VARIATION EXPERIMENTS WITH DITHIZONE

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ABSTRACT

The well-known color development dithizone reagent, H_2Dz, has been used extensively in trace metal analyses for almost a century. However, very little effort has gone into developing derivatives for aqueous media applications in absence of organic solvents. Synthesis of a suitable (p-COOH)H_2Dz derivative is reported here. For spectrophotometric determinations of various metal (M) to ligand (L) ratio's in dithizonato metal complexes this reagent provides a versatile alternative for undergraduate chemistry practicals that is cost-effective, yielding repeatable results. The mole-ratio method yield a ratio of 1M : 1L for the silver dithizonate complex and 1M : 3L for cobalt. Employing the continuous variation method give M : L ratio's of 1 : 3 for both nickel and cobalt. Formation constants are readily calculated from absorbance data. Complete methods, data, calculations and outcomes are reported here. All ratio's are confirmed by mass spectroscopy. *[African Journal of Chemical Education—AJCE 7(2), July 2017]*

Graphical Abstract
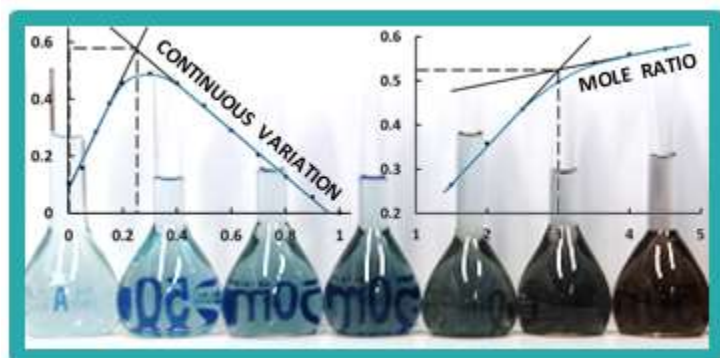
INTRODUCTION

Metal to ligand ratios and constants of formation are important aspects to consider during selection of suitable complexing reagents. Undergraduate analytical chemistry textbooks like Fundamentals of Analytical Chemistry describe three methods traditionally used for determining metal to ligand ratios in complexes [1]:

1.   the continuous variation method,

2.   the mole ratio method and

3.   the slope-ratio method.

Without having to isolate complexes as pure compounds, both its composition and formation constants, K_f, may be determined by means of spectrophotometrical methods. This is possible since measurements are recorded quantitatively without disturbing reaction equilibria. Species that do not absorb are also readily determined, typically by using color development reagents like thiocyanate, 1,10-phenanthroline, or as reported in the present study, by reaction with the intensely colored blue-green carboxydithizone, henceforth referred to as the ligand, L.

Yoe and Jones introduced the mole ratio method, which is ideally suited for determination of complex stoichiometry [2-3]. Absorbances are measured for a series of solutions where in the metal concentration is kept constant while that of the ligand is systematically increased. Resulting plots of absorbance *versus* mole-ratio of reactants are characterized by two straight lines of different slopes that intersect at the equivalence point mole ratio, see Figures 1 and 2. Not just one, but also the formation of two or more complexes may simultaneously be identified, i.e. where successive slope changes occur, complexes have different colors or molar absorptivities, and constants of formation differ amply.

Formation constants may be obtained from absorbance data at the curved section of the plot. An increase in curvature is indicative of weaker complexes and therefore smaller constants of formation, which inevitably also increases uncertainty during identification of mole ratios [4]. Limiting factors are the stability of the formed complex, effect of the buffer and presence of masking reagents [5]. Techniques that may enhance application of this method to weak complexes are discussed by Chriswell *et al* [6].

It was however Job who originally introduced the method for determining formulas and formation constants of complexes [7-8]. The method was later modified, becoming the most widely used routine for determining composition of soluble metal complexes. Job's method later commonly became known as the continuous variation method. A series of solutions are prepared where in the concentration of one reactant is systematically increased while the other is decreased; the combined concentration staying constant throughout. Solution absorbances are subsequently recorded at an appropriate wavelength. Provided that only a single complex forms, the plot of absorbance values against the volume fraction of one reactant is typically triangular in shape. Curvature at the apex is indicative of a complexation reaction that does not proceed to completion, see Figures 3 and 4.

In practical terms, the corresponding K_f may be determined from the difference in absorbance between the said apex, and the intersect of two theoretical straight lines on both sides of the curvature that are extrapolated along the linear parts of the plot. Certainty in results is obtained only when one predominant complex forms, where the reactants and complex are not involved in associated equilibria [9].

A typical example where Job's method is often applied is the iron-thiocyanate system. It however yields a variety of coordination ratio's, depending on sometimes minute changes in

reaction conditions, like pH [10]. We found that results are consequently inconsistent amongst large groups of relatively inexperienced students, which poses a major challenge during undergraduate practicals. We therefore developed a new system utilizing a dithizone derivative that may complex several metals while requiring little additional reactants or costly organic solvents, see Scheme 1. Hereby the focus remains on the spectrophotometric method and not on synthesis. The carboxy derivative of dithizone unfortunately is not yet available commercially, but may readily be synthesized in large quantities and in high yield within less than a day. Due to the very intense color of dithizone only minute quantities are required during experiments employing both the mole ratio and continuous variation methods. For a student to execute and complete any one of the four experimental procedures reported here below, together with its set of calculations, is not expected to take more than 3 hours.
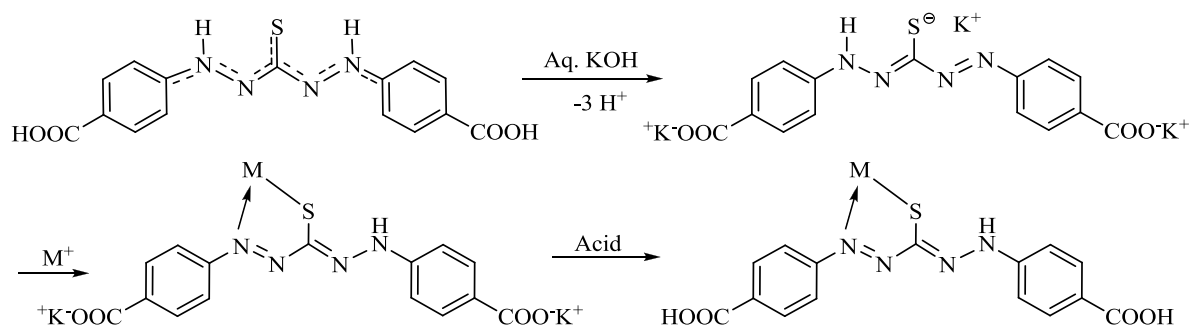
Scheme 1. Aqueous medium metal complexation with para-carboxydithizone.

Ever since the early 1900's when Hellmut Fischer [11] introduced dithizone as new organic reagent in the field of analytical chemistry it has had a great impact on analytical procedures. These include various titrimetry applications, as well as mono- and mixed-color procedures applicable to trace metal analyses. Special features are its great sensitivity and considerable selectivity, the intensely colored complexes it forms with metals, its solubility in a range of organic solvents, and insolubility in water while readily soluble in alkali water. These properties are

essential during metal extractions or analyses. As the availability of spectrometers increased, former procedures were refined and improved, especially also with regard to the determination of toxic metals, which stays an important sub-discipline of analytical chemistry [12]. Having done much experimental work himself, Irving wrote comprehensive reviews on many aspects of dithizone chemistry [13-14]. A single mole-ratio study involving dithizone was reported by him in 1952; a mono-phase consisting of 20 % chloroform, 70 % ethanol and 10 % water was used to confirm 1M : 2L complex formation in zinc(II) dithizonate [15]. More recently, extensive structural,**Error! Bookmark not defined.** [16] electrochemical [17-18], femtosecond laser spectroscopy [19-20], syntheses and photo-isomerization kinetics work [21-22] involving the dithizone ligand were reported.

After years of experience with typical related spectrometry challenges faced by students, the present chemical systems had been researched and developed, supplying a new variety of options from which the lecturer may choose. Best results are presented; Ag and Co for the mole ratio method and Ni and Co for the continuous variation method. The larger pool from which the selection was made may be viewed under Supporting Information.

Employing dithizone may perhaps have the disadvantage of both the ligand and the product absorbing strongly in the UV-visible spectral region. However, since only changes in absorbance are measured here, interference by other absorbing species is not effecting experimental outcome. Nevertheless, where possible, absorbances of complex products are obtained in non-overlapping regions of spectra. In addition, absorbance data at equivalence points only, i.e. where no species are in excess, are considered for calculating molar absorptivities, concentrations and formations constants.

Although unsubstituted dithizone itself is also soluble in aqueous alkali medium, it is not nearly as soluble as that of the –COOH derivative, which latter compound also proves to be more stable in solution. With both potassium and ammonium hydroxide being considered as bases, variation in outcome is however observed. In this regard, it should be brought to mind that ammonia itself may also act as a ligand and thus interfere during complexation reactions. High base concentration on the one hand improves ligand solubility, while on the other hand decreases stability.

Particular care is taken to weigh accurately as to obtain identical M and L concentrations where needed. Preparation of the ligand solutions are done immediately prior to experiment, as to prevent concentration and thus absorbance changes that may occur over long periods of time. For the sake of convenience and consistency, both the reagent and metal solutions may be added from either two burettes or dispenser pipettes. Clean and dry glassware are of course required at all times.

EXPERIMENTAL

Solvents and reagents are supplied by Sigma-Aldrich and Merck and used without further purification.   Distilled water, free of interfering metals, is used.   A Shimadzu UV-2550 spectrophotometer and glass cuvettes is used for UV-visible spectrophotometric measurements. All measurements are within absorbance ranges that obey Beer's law. Significant figures are used and shown in all calculations.

Synthesis [22]

(p-COOH)nitroformazan: An acidic mixture of concentrated hydrochloric acid (30 mL) and water (50 mL) is used to dissolve 4-aminobenzoic acid (8.2170 g, 60 mmol) in a 250 mL

beaker placed on a cooling stirrer (or ice bath) and kept below 0 °C. While stirring, sodium nitrite (6.2186 g, 90 mmol) is added in small portions as to prevent the temperature from rising above 0 °C, forming the creamy yellow diazo solution. This solution is then added to a well stirred solution of sodium acetate trihydrate (100 g), glacial acetic acid (60 mL) and water (40 mL) in a 500 mL beaker. (Note: Vigorously stir the salt before and while adding the water-acid mixture or *vice versa*, else dissolution may not be achieved.) Nitromethane (17 mL, 300 mmol) is added to the reaction mixture, stirred at room temperature until a brick red slurry is obtained, while water is periodically added as the reaction mixture thickens. The formazan is filtered off and washed with copious amounts of cold water and dried in an open fume hood or oven at 60 °C.

(*p*-COOH)nitroformazan: orange-red (9.7 g, 93 %). M.p. 210 °C, UV-vis (acetone) λ_{max} 449 nm. *(p-COOH)thiocarbazone:* Nitroformazan (4 g, 12 mmol) is dissolved in absolute ethanol (200 mL) in a lightly stoppered Erlen Meyer flask. Ammonium sulphide (25 mL, 70 mmol, 20 %) is added and stirred at *ca* 30 °C for 30 minutes. After 20 minutes a light yellow precipitate (thiocarbazone) forms, which is filtered off in a sintered glass funnel. The funnel containing the product is placed in a clean beaker and potassium hydroxide solution (250 mL, 2 %) is poured onto the precipitate in the funnel while stirring with a spatula to aid dissolution. The yellow thiocarbazone precipitate, (*p*-COOH)H_4Dz, is hereby oxidized to the dark red potassium salt (K^+HDz$^-$) solution.

(p-COOH)dithizone: Dilute HCl (250 mL, 2 %) is cooled down to < 5 °C and slowly poured into the deep orange-red dithizonate solution until the green-black dithizone, (*p*-COOH)H_2Dz, is fully precipitated. The product mixture is centrifuged (or filtered) and washed with ample amounts of cold water (< 5 °C). For purification purposes, the former base-acid steps are repeated three times, i.e. by re-dissolving the precipitate in 2% aqueous KOH solution (200

mL) followed by addition of cold dilute HCl. The product is dried in a fume hood and washed with

warm DCM (100 mL × 3). (*p*-COOH)dithizone: black (3 g, 73 %), M.p. 199 °C, UV-vis (acetone)

λ_{max} 452 and 634 nm.

   Metal complexation: Not all metals readily react with the neutral dithizone ligand. Fast

complexation is promoted by addition of base which singly deprotonates the dithizone backbone,

see Scheme 1. (For Ag, Co, Ni, Pb and Hg complex syntheses and characterization data, see

Supporting Information.)

Mole Ratio Method
1. $Ag + xL \rightarrow AgL_x$

Two standard solutions (5.1×10^{-4} M, 100.0 mL) are prepared by dissolving:
- 0.0087 g AgNO$_3$ (5.1×10^{-5} mole, 169.9 g/mol) in distilled water, and
- 0.0176 g L (5.10×10^{-5} mole, 344.4 g/mol) in 0.1 M NH$_4$OH (*aq*).
   To each of nine 50.0 mL volumetric flasks 2.50 mL of the silver solution is added, followed

by 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00 and 4.50 mL respectively of the L solution, see

Table 1. The flasks are filled to the mark with 0.1 M NH$_4$OH (*aq*). The silver concentration in all

solutions is 2.6×10^{-5} M. Thirty minutes are allowed, with occasional swirling, for the reaction to

complete before absorbance readings are obtained at 590 nm.

Table 1. Absorbance of silver solutions with varied L concentrations.

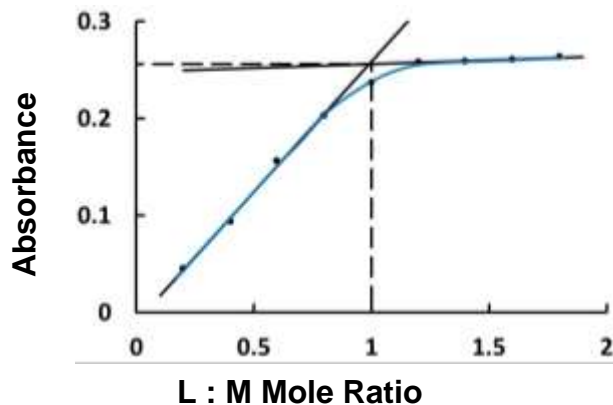| Volume L (mL)	L : M	Absorbance
0.50	0.2	0.045
1.00	0.4	0.093
1.50	0.6	0.158
2.00	0.8	0.203
2.50	1.0	0.237
3.00	1.2	0.261
3.50	1.4	0.258
4.00	1.6	0.260
4.50	1.8	0.263

Figure 1. Complexation reaction of Ag^+ with $(p\text{-COOH})H_2Dz$: mole ratio plot of absorbance *vs* mole L per mole Ag^+ cation, in 0.1 M NH_4OH (*aq*) at 590 nm. Trendlines fitted to linear sections intersect at 1M : 1L

From the mole ratio plot in Figure 1 on the *x*-axis, it is seen that Ag^+ reacts with one ligand. This observation is expected, being consistent with the monovalent oxidation state of the silver cation.

Both Pb^{2+} and Hg^{2+} also give a 1M : 1L ratio for this particular ligand, although being divalent cations. (See Supporting Information) This could however not be confirmed by other instrumental techniques, eg. no specific ratio could be derived from mass spectra. Nevertheless, instead of the more costly silver cation, lead may be used as an example of a 1M : 1L complex.

At the equivalence point on the *y*-axis a difference is observed between the absorbance, $A_{th} = 0.26$, of the theoretically fully complexed species (trendline intersect), and $A_{exp} = 0.24$, the corresponding experimental value (curve bend). From this data the formation constant, K_f, is calculated for the reaction,

$$Ag^+ + L^- \overset{K_f}{\rightleftharpoons} AgL$$

$$\text{where } K_f = \frac{[AgL]}{[Ag][L]} \quad - \quad (1)$$
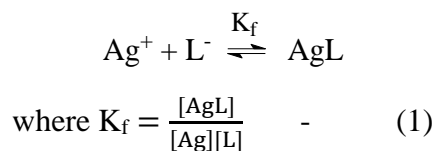
The experimental AgL concentration, C_{exp}, is calculated from the Beer law equation,

$$A = \varepsilon bC \quad - \quad (2)$$

where $A_{th} = 0.26$ (reaction complete), $b = 1.00$ cm (cuvette path length) and

$$C_{th} = \frac{5.1 \times 10^{-4} \text{ M} \times 2.50 \text{ mL}}{50.0 \text{ mL}}$$

$$= 2.6 \times 10^{-5} \text{ M}$$

Thus, the molar absorptivity,

$$\varepsilon = \frac{0.26}{2.6 \times 10^{-5} \text{ M} \times 1.00 \text{ cm}}$$

$$= 10\ 000 \text{ L mol}^{-1} \text{ cm}^{-1} \text{ at 590 nm}$$

From equation 2

$$C_{exp} = \frac{0.24}{10\ 000 \text{ L mol}^{-1} \text{ cm}^{-1}}$$

$$= 2.4 \times 10^{-5} \text{ M}$$

Due to the 1M : 1L reaction stoichiometry, unreacted

$$[Ag] = [L]$$
$$= [AgL]_{th} - [AgL]_{exp}$$
$$= 2.6 \times 10^{-5} \text{ M} - 2.4 \times 10^{-5} \text{ M}$$
$$= 2.0 \times 10^{-6} \text{ M}$$

From equation 1

$$K_f = \frac{[2.4 \times 10^{-5} \text{ M}]}{[2.0 \times 10^{-6} \text{ M}][2.0 \times 10^{-6} \text{ M}]}$$

$$= 6.0 \times 10^{6} \text{ M}^{-1}$$

2. $Co + xL \rightarrow CoL_x$

Two standard solutions (5.10×10^{-4} M, 100.0 mL) are prepared by dissolving:

- 0.0121 g $CoCl_2 \cdot 6H_2O$ (5.10×10^{-5} mole, 237.9 g/mol) in distilled water, and

- 0.0176 g L (5.10×10^{-5} mole, 344.4 g/mol) in 0.1 M KOH (*aq*).

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To each of seven 50.0 mL volumetric flasks 2.00 mL of the cobalt solution is added,

followed by 3.00, 4.00, 5.00, 6.00, 7.00, 8.00 and 9.00 mL respectively of the L solution, see Table

2. The flasks are filled to the mark with 0.1 M KOH (*aq*). The cobalt concentration in all solutions

is 2.04×10^{-5} M. Before absorbance readings are obtained 30 minutes are allowed, with occasional

swirling, for the reaction to complete.

Table 2. Absorbance of cobalt solutions with varied L concentrations.

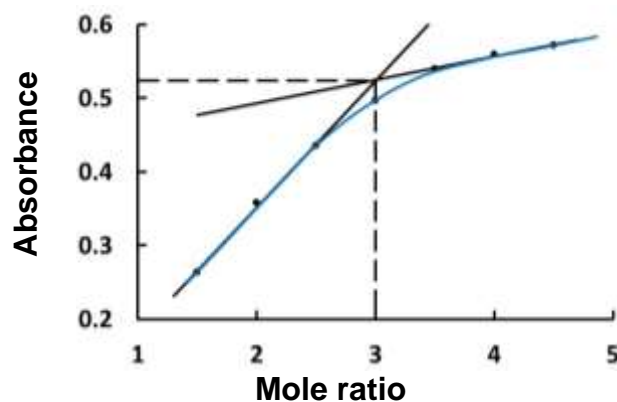| Volume L (mL)	L : M	Absorbance
3.00	1.5	0.263
4.00	2.0	0.358
5.00	2.5	0.435
6.00	3.0	0.497
7.00	3.5	0.540
8.00	4.0	0.559
9.00	4.5	0.572

Figure 2. Complexation reaction of Co^{2+} with (*p*-COOH)H_2Dz: mole ratio plot of absorbance *vs* mole L per mole Co^{2+} cation, in 0.1 M KOH (*aq*) at 490 nm. Trendlines fitted to linear sections intersect at 1M : 3L.

From the mole ratio plot in Figure 2 it is seen that Co^{2+} reacts with three ligands. This is

unexpected, however, it was recently reported elsewhere that Co^{2+} undergoes fast auto-oxidation

during this reaction, yielding the Co(III) complex [23].

At reaction completion, $A_{th} = 0.520$, while observed $A_{exp} = 0.497$.

(Calculations are related to the Ag mole ratio experiment.)

$$K_f \quad = \frac{[CoL_3]}{[Co][L]^3} \quad \text{for the reaction}$$

$$Co^{2+} + 3L^- \underset{}{\overset{K_f}{\rightleftharpoons}} CoL_3$$
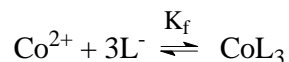
The theoretical concentration at reaction completion,

$C_{th} =$ 2.04×10^{-5} M, which gives

$\varepsilon \ =$ $25\ 500$ L mol^{-1} cm^{-1} at 490 nm

Thus, for the CoL_3 complex

C_{exp} $=$ 1.95×10^{-5} M

The 1M : 3L reaction stoichiometry results in unreacted

$[Co] =$ $\frac{1}{3}[L]$

         $=$ $[CoL_3]_{th} - [CoL_3]_{exp}$

         $=$ 9.00×10^{-7} M

Thus,

$K_f =$ $\dfrac{[1.95 \times 10^{-5} \text{ M}]}{[9.00 \times 10^{-7} \text{ M}][3 \times 9.00 \times 10^{-7} \text{ M}]^3}$

        $=$ 1.1×10^{18} M^{-3}

Continuous Variation Method

1. Ni + xL → NiL$_x$

Two standard solutions (5.10×10^{-4} M, 100.0 mL) are prepared by dissolving:

- 0.0134 g NiSO$_4$·6H$_2$O (5.10×10^{-5} mole, 262.8 g/mol) in distilled water, and

- 0.0176 g L (5.10×10^{-5} mole, 344.4 g/mol) in 0.1 M KOH (*aq*).

To fifteen 50.0 mL volumetric flasks 0.00, 0.20, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, 9.00 and 10.00 mL of the nickel solution is added, and also 10.00, 9.80,

9.50, 9.00, 8.50, 8.00, 7.50, 7.00, 6.00, 5.00, 4.00, 3.00, 2.00, 1.00 and 0.00 mL of the L solution,

see Table 3. The flasks are filled to the mark with 0.1 M KOH (*aq*). Before absorbance readings

are obtained 30 minutes are allowed, with occasional swirling, for the reaction to complete.

Table 3. Mixing ratio and absorbance of nickel and ligand solution mixtures.

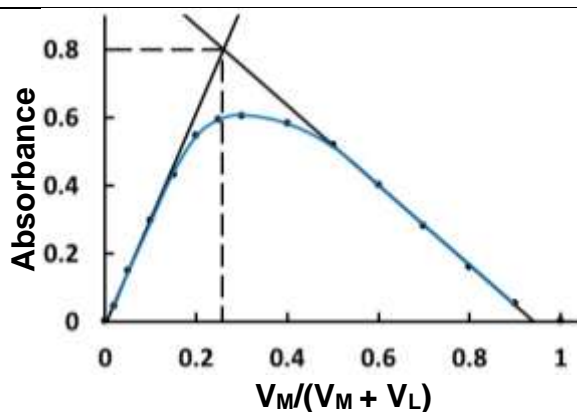| Volume M (mL)	Volume L (mL)	$\dfrac{V_M}{V_M + V_L}$	Absorbance
0.00	10.00	0.00	0.003
0.20	9.80	0.02	0.046
0.50	9.50	0.05	0.149
1.00	9.00	0.10	0.297
1.50	8.50	0.15	0.432
2.00	8.00	0.20	0.546
2.50	7.50	0.25	0.595
3.00	7.00	0.30	0.606
4.00	6.00	0.40	0.583
5.00	5.00	0.50	0.521
6.00	4.00	0.60	0.403
7.00	3.00	0.70	0.281
8.00	2.00	0.80	0.161
9.00	1.00	0.90	0.054
10.00	0.00	1.00	0.001

Figure 3. Complexation reaction of Ni^{2+} with (*p*-COOH)H_2Dz: continuous variation plot of absorbance *vs* volume fraction, in 0.1 M KOH (*aq*), at 690 nm. Trendlines fitted to linear sections intersect at 1M : 3L.

From the continuous variation plot in Figure 3 it is seen that Ni^{2+} reacts with three ligands. At the equivalence point the V_M to V_L ratio is *ca* 2.5 : 7.5. With both the M and L solutions having similar concentrations, the mole quantity ratio is therefore also 1 : 3.

This unexpected coordination ratio is confirmed by mass spectroscopy. Additional experiments, which goes beyond the scope of this report, indicate the stable Ni(II) oxidation state persists also in the complex product where coordination by the third ligand is ascribed to dative covalent bonding. Unsubstituted dithizone, which does not have the added two –COOH coordination sites, reacts with Ni^{2+} to give the NiL_2 complex [24]. (See Supporting Information for additional information.)

At the equivalence point in Figure 3 on the *y*-axis a difference is observed between the absorbance, $A_{th} = 0.80$, of the theoretically fully complexed species (trendline intersect), and $A_{exp} = 0.60$, the corresponding experimental value (curve bend). From this data the formation constant, K_f, is calculated for the reaction,

$$Ni^{2+} + 3L^- \xrightleftharpoons{K_f} NiL_3$$

$$\text{where } K_f = \frac{[NiL_3]}{[Ni][L]^3} \quad - \quad (1)$$
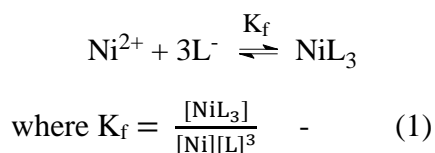
The experimental NiL_3 concentration, C_{exp}, is calculated from the Beer law equation,

$$A = \varepsilon bC, \quad - \quad (2)$$

where $A_{th} = 0.80$ (reaction complete), $b = 1.00$ cm (cuvette path length) and

$$C_{th} = \frac{5.10 \times 10^{-4} \text{ M} \times 2.50 \text{ mL}}{50.0 \text{ mL}}$$
$$= 2.55 \times 10^{-5} \text{ M}$$

Thus, the molar absorptivity

$$\varepsilon = \frac{0.80}{2.55 \times 10^{-5} \text{ M} \times 1.00 \text{ cm}}$$
$$= 31\,000 \text{ L mol}^{-1} \text{ cm}^{-1} \text{ at 700 nm.}$$

From equation 2

$$C_{exp} = \frac{0.60}{31\,000 \text{ L mol}^{-1} \text{ cm}^{-1}}$$
$$= 1.9 \times 10^{-5} \text{ M}$$

The 1M : 3L reaction stoichiometry results in unreacted

$$[Ni] = \tfrac{1}{3}[L]$$
$$= [NiL_3]_{th} - [NiL_3]_{exp}$$
$$= 2.55 \times 10^{-5} \text{ M} - 1.9 \times 10^{-5} \text{ M}$$
$$= 6.5 \times 10^{-6} \text{ M}$$

From equation 1

$$K_f = \frac{[1.9 \times 10^{-5} \text{ M}]}{[6.5 \times 10^{-6} \text{ M}][3 \times 6.5 \times 10^{-6} \text{ M}]^3}$$
$$= 3.9 \times 10^{14} \text{ M}^{-3}$$

2. $Co + xL \rightarrow CoL_x$

Two standard solutions (5.10×10^{-4} M, 100.0 mL) are prepared by dissolving:

- 0.0121 g $CoCl_2 \cdot 6H_2O$ (5.10×10^{-5} mole, 237.93 g/mol) in distilled water, and

- 0.0176 g L (5.10×10^{-5} mole, 344.35 g/mol) in 0.1 M NH_4OH (*aq*).

To thirteen 50.0 mL volumetric flasks 0.00, 0.50, 1.00, 1.50, 2.00, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, 9.00 and 10.00 mL of the cobalt solution is added, and also 10.00, 9.50, 9.00, 8.50, 8.00, 7.00, 6.00, 5.00, 4.00, 3.00, 2.00, 1.00 and 0.00 mL of the L solution, see Table 4. The flasks are filled to the mark with 0.1 M NH_4OH (*aq*). Before absorbance readings are obtained 30 minutes are allowed, with occasional swirling, for the reaction to complete.

Table 4. Mixing ratio and absorbance of cobalt and ligand solution mixtures.

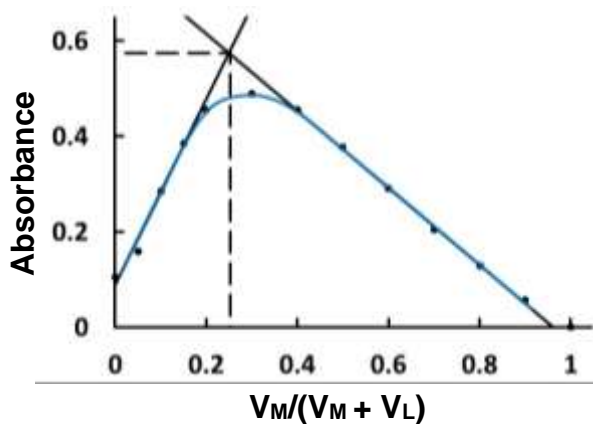| Volume M (mL)	Volume L (mL)	$\dfrac{V_M}{V_M + V_L}$	Absorbance
0.00	10.00	0.00	0.104
0.50	9.50	0.05	0.160
1.00	9.00	0.10	0.285
1.50	8.50	0.15	0.386
2.00	8.00	0.20	0.455
3.00	7.00	0.30	0.490
4.00	6.00	0.40	0.455
5.00	5.00	0.50	0.378
6.00	4.00	0.60	0.291
7.00	3.00	0.70	0.205
8.00	2.00	0.80	0.129
9.00	1.00	0.90	0.056
10.00	0.00	1.00	0.000

Figure 4. Complexation reaction of Co^{2+} with (p-COOH)H_2Dz: continuous variation plot of absorbance *vs* volume fraction, in 0.1 M NH_4OH (*aq*) at 600 nm. Trendlines fitted to linear sections intersect at 1M : 3L.

From the continuous variation plot in Figure 4 it is seen that, as for Ni^{2+}, the Co^{2+} cation also reacts with three ligands. The ratio is determined as for Ni here above. This result is of course consistent with what was observed in the Co mole ratio experiment.

At reaction completion, $A_{th} = 0.580$, while observed $A_{exp} = 0.480$.

$$K_f = \frac{[CoL_3]}{[Co][L]^3} \quad \text{for the reaction}$$

$$Co^{2+} + 3L^- \xrightleftharpoons{K_f} CoL_3$$
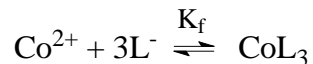
The theoretical concentration at reaction completion,

$$C_{th} = 2.55 \times 10^{-5} \text{ M, and}$$

$$\varepsilon = 23\,000 \text{ L mol}^{-1} \text{ cm}^{-1} \text{ at 600 nm.}$$

Thus, for the CoL_3 complex

$$C_{exp} = 2.1 \times 10^{-5} \text{ M}$$

The 1M : 3L reaction stoichiometry results in unreacted

$$[Co] = {}^1/_3[L]$$
$$= [CoL_3]_{th} - [CoL_3]_{exp}$$
$$= 4.5 \times 10^{-6} \text{ M}$$

Thus,

$$K_f = \frac{[2.1 \times 10^{-5} \text{ M}]}{[4.5 \times 10^{-6} \text{ M}][3 \times 4.5 \times 10^{-6} \text{ M}]^3}$$
$$= 1.9 \times 10^{15} \text{ M}^{-3}$$

CONCLUSION

Utilization of the dithizone ligand derivitized with carboxy substituents on the *para* positions of the two phenyl rings provides reliable and uncomplicated alternatives for teaching undergraduate students some of the neat applications of UV-visible spectrophotometry. While not requiring the usual wet chemistry associated with other chemical systems that often prevent students from reaching the desired results for which such experiments are primarily designed, the

present system maintains focus on the physical spectroscopy technique. The use of aqueous media is an important cost incentive.

The larger formation constant of cobalt (1.1×10^{18} M^{-3}) resulting from the mole ratio study as compared to the continuous variation study (1.9×10^{15} M^{-3}), is ascribed to the different bases that were used. Ammonia itself acts as a ligand which competes with dithizone during the complexation reaction,[Error! Bookmark not defined.] and thus also in the present continuous variation experiment. The use of potassium hydroxide in the mole ratio experiment, on the contrary, facilitates a more complete reaction, and thus a larger constant of formation. Although performed in the presence of KOH, the formation constant of NiL_3 (3.9×10^{14} M^{-3}) is almost one order of magnitude smaller than that of CoL_3 in the presence of ammonia. K_f for AgL is 6.0×10^6 M^{-1}, which is the least stable complex in the present series. NH_4OH is used in the Ag mole ratio experiment.

Observed metal to ligand ratio's, i.e. AgL, CoL_3 and NiL_3, are all confirmed by mass spectroscopy and/or X-ray crystallography.

From a variety of combinations that were tested, the above four experiments yield best results.

ASSOCIATED CONTENT
Supporting Information.
Synthesis method and characterization data for the complexes; Co, Ni, Pb, Ag and Hg, with dithizone and *para*-carboxydithizone.

UV-visible spectra of the free ligand, *p*-COOH–H_2Dz, in various solvents.
UV-visible overlay spectra of Co, Ni, Pb & Ag complexes.

Spectral change of the Co complex in methanol over time.

Mole ratio and continuous variation methods, photo's, overlay spectra, data and plots for the various metal complexes, ligands and bases, as well as brief discussions or motivations where necessary.

This material is available free of charge via the Internet at http://pubs.acs.org.

Supporting Information

Synthesis

Adapted method as reported in

(Von Eschwege, K. G. *et al.* Intramolecular interactions in a new *tris*-dithizonatocobalt(III) complex, *Electrochimica Acta*, **2013**, 112, 747–755.

doi.org/10.1016/j.electacta.2013.08.192

Von Eschwege, K. G.; Van As, L.; Swarts, J. C. Electrochemistry and spectro-electrochemistry of dithizonatophenylmercury(II), *Electrochimica Acta*, **2011**, 56, 10064-10068.

doi.org/10.1016/j.electacta.2011.08.094)

tris-(*p*-COOH)dithizonatocobalt(III)

(*p*-COOH)H_2Dz (0.1520 g, 0.44 mmol) was dissolved in 50 mL aqueous KOH (0.5689 g, 10 mmol) solution, in a 250 mL beaker equipped with a magnetic stirrer. Cobalt(II) chloride hexahydrate, $CoCl_2 \cdot 6H_2O$, (0.0320 g, 0.13 mmol,) in 50 mL water was added to the dithizonate solution. The reaction mixture was stirred for 1 hour at room temperature, to form a deep blue solution. Glacial acetic acid (20 mL) was added to precipitate out the brown black product, and the precipitate was recovered by using a centrifuge. Acid-base purification was repeated three times by repeatedly dissolving the precipitate in 0.1 M aqueous KOH and again precipitating it out by addition of 20 mL glacial acetic acid.

Co(*p*-COOH-HDz)$_3$: (0.1417 g, 89 %). M.p. > 360 ^{o}C. UV-vis (methanol) λ_{max} 318 and 567 nm. δ_H (600 MHz, DMSO-d$_6$)/ppm: 7.5 – 8.2 (24$\underline{H}$, Ar-$\underline{H}$'s, 6 × C$_6\underline{H_4}$).

Tris-dithizonatocobalt(III)

Dithizone, H_2Dz, (0.2520 g, 0.98 mmol) was dissolved in 10 mL aqueous KOH (0.5689 g, 10 mmol) solution in a 250 mL beaker equipped with a magnetic stirrer, then gradually increase the volume up to 50 mL with water. Cobalt(II) chloride hexahydrate, $CoCl_2 \cdot 6H_2O$, (0.0752 g, 0.31

mmol) was added to the dithizonate solution and the resultant reaction mixture was stirred at room temperature for 1 hour. The product was extracted with 100 mL DCM and washed with 100 mL 0.1 M aqueous KOH until the aqueous layer was colorless. The extract was washed with 100 mL water and dried overnight in the fume hood.

Co(HDz)$_3$: (0.2163 g, 80 %). M.p. 175 ^{o}C, UV-vis (methanol) λ_{max} 448 and 556. δ_H (600 MHz, CDCl$_3$)/ppm: 6.4 – 7.45 (30$\underline{H}$, Ar-$\underline{H}$'s, 6 × C$_6\underline{H_5}$), 9.49 (3H, s, 3 × N$\underline{H}$).

tris-(*p*-COOH)dithizonatonickel(II)

(*p*-COOH)H$_2$Dz (0.1514 g, 0.44 mmol) was reacted with nickel(II) sulphate hexahydrate, NiSO$_4$·6H$_2$O, (0.0364 g, 0.14 mmol).

Ni(*p*-COOH-HDz)$_3$: (0.1518 g, 95 %). M.p. > 360 ^{o}C, UV-vis (methanol). λ_{max} 315 and 458 nm. δ_H (600 MHz, DMSO-d$_6$)/ppm: 7.1 – 8.3 (24$\underline{H}$, Ar-$\underline{H}$'s, 2 × C$_6\underline{H_4}$).

bis-dithizonatonickel(II)

H$_2$Dz (0.2519 g, 0.98 mmol) and nickel(II) sulphate hexahydrate, NiSO$_4$·6H$_2$O, (0.1122 g, 0.41 mmol) were reacted.

Ni(HDz)$_2$: (0.2408 g, 86 %). M.p. 230 ^{o}C, UV-vis (acetone) λ_{max} 279, 456, 560 and 686 nm. δ_H (600 MHz, DMSO-d$_6$)/ppm: 6.3 – 7.4 (20$\underline{H}$, Ar-$\underline{H}$'s, 2 × C$_6\underline{H_5}$).

(*p*-COOH)dithizonatolead(II)

(*p*-COOH)H$_2$Dz (0.1533 g, 0.45 mmol) was dissolved in 0.1 M aqueous NH$_4$OH and reacted with lead(II) acetate trihydrate, Pb(CH$_3$COO)$_2$·3H$_2$O, (0.1743 g, 0.46 mmol).

Pb(*p*-COOH-HDz): (0.1825 g, 74 %). M.p. > 360 ^{o}C, UV-vis (methanol) λ_{max} 300 and 507 nm. δ_H (600 MHz, DMSO-d$_6$)/ppm: 7.4 – 8.2 (8$\underline{H}$, Ar-$\underline{H}$'s, 2 × C$_6\underline{H_4}$), 10.3 (1H, s, 2 × COO$\underline{H}$).

bis-dithizonatolead(II)

H$_2$Dz (0.1282 g, 0.50 mmol) was dissolved in 0.1 M aqueous NH$_4$OH and reacted with lead(II) acetate, Pb(CH$_3$COO)$_2$·3H$_2$O, (0.0684 g, 0.26 mmol).

Pb(HDz)$_2$: (0.1454 g, 81 %). M.p. 210 ^{o}C, UV-vis (methanol) λ_{max} 257 and 505 nm. δ_H (600 MHz, DMSO-d$_6$)/ppm: 6.9 – 7.9 (20$\underline{H}$, Ar-$\underline{H}$'s, 4 × C$_6\underline{H_5}$), 10.00 (2H, s, 2 × N$\underline{H}$).

(*p*-COOH)dithizonatosilver(I)

(*p*-COOH)H_2Dz (0.1530 g, 0.45 mmol) was dissolved in 0.1 M aqueous NH_4OH and reacted with silver nitrate, $AgNO_3$, (0.0783 g, 0.46 mmol).

Ag(*p*-COOH-HDz): (0.1790 g, 88 %). M.p. 320 ºC, UV-vis (methanol) λ_{max} 314 and 474 nm. δ_H (600 MHz, DMSO-d_6)/ppm: 7.4 – 8.1 (8$\underline{H}$, Ar-$\underline{H}$'s, 2 × $C_6\underline{H}_4$), 11.07 (1H, s, 1 × N$\underline{H}$), 12.6 (2H, s, 2 × COO$\underline{H}$).

dithizonatosilver(I)

H_2Dz (0.1302 g, 0.5 mmol) and silver nitrate, $AgNO_3$, (0.0867 g, 0.51 mmol) were reacted. Silverdithizonate: (0.1700 g, 94 %). M.p. 160 ºC, UV-vis (methanol) λ_{max} 253 and 490 nm nm. δ_H (600 MHz, DMSO-d_6)/ppm: 6.9 – 7.8 (10$\underline{H}$, Ar-$\underline{H}$'s, 2 × $C_6\underline{H}_5$), 10.38 (1H, s, 1 × N$\underline{H}$).

(*p*-COOH)dithizonatophenylmercury(II)

PhHg(*p*-COOH-HDz): dark red (0.9678 g, 85 %). M.p. 280 ºC, UV-vis (methanol) λ_{max} 293 and 485 nm. δ_H (600 MHz, DMSO-d_6)/ppm: 7.2 – 8.1 (13H, Ar-$\underline{H}$'s, 1 × $C_6\underline{H}_5$ and 2 × $C_6\underline{H}_4$), 11.0 (1H, s, 2 × N$\underline{H}$). Product photochromic.
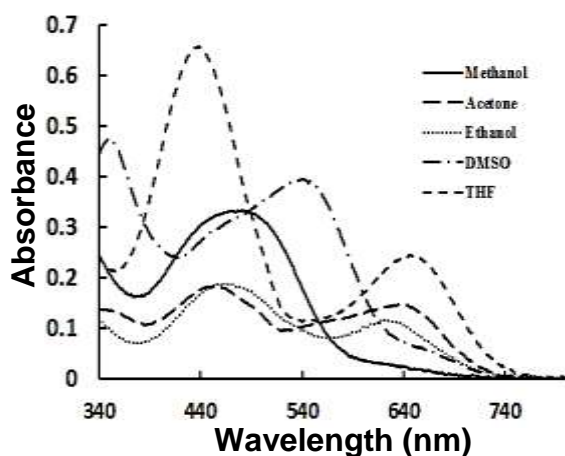
Figure SI-1. 3.23 x 10^{-5} M *p*-COOH–H_2Dz in solvents with dielectric constants above 20.

It should be noted that p-COOH–H2Dz is, amongst others, solvatochromic, concentratochromic and halochromic (pH dependant).

NB - Care must therefore be taken when solvents or concentrations, etc. are altered, as absorbance spectra may change completely and thus affect experimental outcomes.
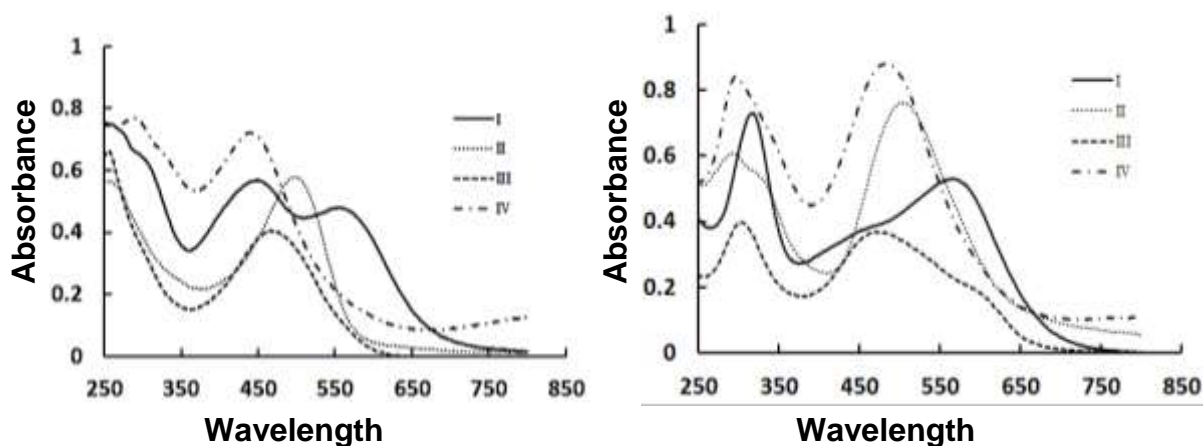
Figure SI-2. Comparative UV-vis spectra of all metal (Co, Ni, Pb and Ag)HDz (left) and *p*-COOH-HDz complexes (right) in methanol. I – Co(HDz)$_3$, II – Pb(HDz)$_2$, III – Ag(HDz), IV – Ni(HDz)$_2$ +NH$_3$ and the same for *p*-COOH-HDz complexes (right).

UV-vis spectra of Co(HDz)$_3$ revealed that there may be some reaction between the complex and methanol, as the absorption maximum is initially only at 550 nm. After some time a second maximum appears at 450 nm. It remains unclear as to what reaction takes place.
The change in spectra over time is illustrated in Figure SI-3.
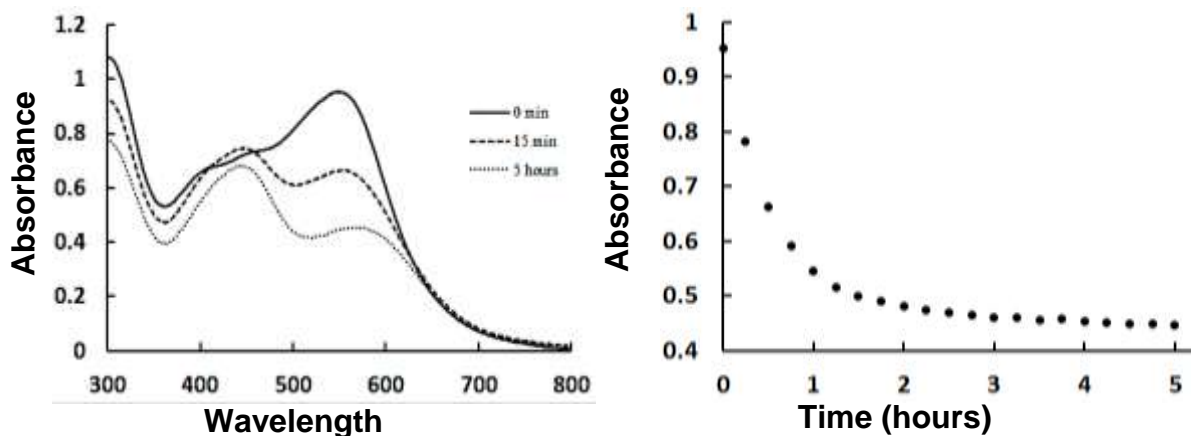
Figure SI-3. UV-vis spectra of Co(HDz)$_3$ at 0 min, 15 min and 5 hours (left) and decay of λ_{max} 550 nm in methanol.

MOLE-RATIO

1.   **Ag(*p*-COOH-HDz) & Ag(HDz)**

5.11 x 10^{-4} M (*p*-COO$^-$)-HDz$^-$ in 0.1 M aqueous NH$_4$OH reacted with an aqueous solution of 5.06 x 10^{-4} M Ag^I from AgNO$_3$ (0.043 g, 2.53 x 10^{-4} moles). To each of the eight 50.0 mL volumetric flasks, 2.50 mL of 5.06 x 10^{-4} M aqueous Ag^I was added and also 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50 and 4.00 mL of 5.11 x 10^{-4} M (*p*-COO$^-$)-HDz$^-$ solution. A time of 40 minutes was allowed, with occasional swirling, for the reaction to complete and the flasks were filled to the mark with aqueous 0.1 M NH$_4$OH.

5.11 x 10^{-4} M HDz$^-$ in 0.1 M aqueous KOH reacted with aqueous of 5.06 x 10^{-4} M Ag^I from AgNO$_3$ (0.043 g, 2.53 x 10^{-4} moles). To each of the seven 50.0 mL volumetric flasks, 4.00 mL of 5.09 x 10^{-4} M aqueous Ag^I was added and also 1.00, 2.00, 3.00, 4.00, 5.00, 6.00 and 7.00 mL of the 5.11 x 10^{-4} M HDz$^-$ solution. A time of 30 minutes was given, with occasional swirling, for the reaction to complete, and the flasks were filled to the mark with aqueous 0.1 M KOH.

Ag(*p*-COOH-HDz) solutions

2.   Pb(p-COOH-HDz) & Pb(HDz)$_2$

5.11 x 10^{-4} M (p-COO$^-$)-HDz$^-$ in 0.1 M of NH$_4$OH was reacted with equimolar solution of aqueous Pb(CH$_3$COO)$_2$·3H$_2$O. To each of the eight 50.0 mL volumetric flasks, 2.50 mL of the 5.11 x 10^{-4} M aqueous Pb^{II} solution was added, and also 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50 and 4.00 mL of the 5.11 x 10^{-4} M HDz$^-$ solution. A time of 30 minutes, with occasional swirling, was allowed for the reaction to complete, and the flasks were filled to the mark with 0.1 M NH$_4$OH solution.

5.11 x 10^{-4} M HDz$^-$ in 0.1 M aqueous NH$_4$OH reacted with an aqueous solution of 5.11 x 10^{-4} M Pb^{II} from Pb(CH$_3$COO)$_2$·3H$_2$O   (0.0121 g, 5.11 x 10^{-5} moles). To each of the seven 50.0 mL volumetric flasks, 2.00 mL of 5.11 x 10^{-4} M aqueous Pb^{II} was added and also 1.00, 1.50, 2.00, 2.50, 3.00, 3.50 and 4.00 mL of the 5.11 x 10^{-4} M HDz$^-$ solution. A time of 30 minutes was given, with occasional swirling, for the reaction to complete, and the flasks were filled to the mark with aqueous 0.1 M NH$_4$OH.

Pb(p-COOH-HDz) solutions

3.   Ni(p-COOH-HDz)$_3$ & Ni(HDz)$_2$

5.11 x 10^{-4} M (p-COO$^-$)-HDz$^-$ in 0.1 M aqueous KOH was reacted with an aqueous solution of 5.10 x 10^{-4} M Ni^{II} from NiSO$_4$·6H$_2$O (0.0134 g, 5.10 x 10^{-4} moles). To each of the seven 50.0 mL volumetric flasks 2.00 mL of 5.10 x 10^{-4} M aqueous Ni^{II} solution was added, and also 3.00, 4.00, 5.00, 6.00, 7.00, 8.00 and 9.00 mL of 5.11 x 10^{-4} M (p-COO$^-$)-HDz$^-$ respectively. A time of 30 min, with occasional swirling, was allowed for the reaction to complete, after which the flask was filled to the mark with aqueous 0.1 M KOH.

5.11 x 10^{-4} M HDz$^-$ in 0.1 M aqueous NH$_4$OH reacted with an aqueous solution of 5.11 x 10^{-4} M Ni^{II} from NiSO$_4$·6H$_2$O (0.0134 g, 5.10 x 10^{-4} moles). To each of the seven 50.0 mL volumetric flasks, 2.00 mL of 5.11 x 10^{-4} M aqueous Ni^{II} was added and also 1.00, 2.00, 3.00, 4.00, 5.00, 6.00 and 7.00 mL of the 5.11 x 10^{-4} M HDz$^-$ solution. A time of 30 minutes was given, with occasional swirling, for the reaction to complete, and the flasks were filled to the mark with aqueous 0.1 M NH$_4$OH.

4.   Co(p-COOH-HDz)$_3$ & Co(HDz)$_3$

p-COOH-H$_2$Dz (0.0176 g, 5.11 x 10^{-5} moles) was dissolved in ca 1 M KOH (0.5626 g in 10 mL) in 100.0 mL volumetric flask and fill up to the mark with water, to obtain a stock solution of 5.11 x 10^{-4} M (p-COO$^-$)-HDz$^-$ in 0.1 M aqueous KOH solution. CoCl$_2$·6H$_2$O (0.0121 g, 5.1 x 10^{-5}

moles) was dissolved in 50 mL water in a 100.0 mL volumetric flask, then filled up to the mark with water to a concentration of 5.09×10^{-4} M. To each of the seven 50.0 mL volumetric flasks 2.00 mL of 5.09×10^{-4} M aqueous Co^{II} solution was added, and also 3.00, 4.00, 5.00, 6.00, 7.00, 8.00 and 9.00 mL of 5.11×10^{-4} M (p-COO⁻)-HDz⁻ respectively. A time of 40 min, with occasional swirling, was allowed for the reaction to complete, after which the flask was filled to the mark with aqueous 0.1 M KOH.

5.11×10^{-4} M HDz⁻ in 0.1 M aqueous KOH reacted with an aqueous solution of 5.09×10^{-4} M Co^{II} from $CoCl_2 \cdot 6H_2O$ (0.0121 g, 5.1×10^{-5} moles). To each of the seven 50.0 mL volumetric flasks, 2.00 mL of 5.11×10^{-4} M aqueous Co^{II} was added and also 3.00, 4.00, 5.00, 6.00, 7.00, 8.00 and 9.00 mL of the 5.11×10^{-4} M HDz⁻ solution. A time of 30 minutes was given, with occasional swirling, for the reaction to complete, and the flasks were filled to the mark with aqueous 0.1 M KOH.

Co(*p*-COOH-HDz)₃ solutions

5.   Hg(*p*-COOH-HDz)

A solution of 5.11×10^{-4} M (p-COO⁻)-HDz⁻ in 0.1 M aqueous NH_4OH reacted with an aqueous solution of 5.08×10^{-4} M Hg^{II} from $HgCl_2$ (0.0138 g, 5.08×10^{-5} moles). To each of the eight 50.0 mL volumetric flasks 2.50 mL of 5.09×10^{-4} M aqueous Hg^{II} was added and also 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50 and 4.00 mL of the 5.11×10^{-4} M (p-COO⁻)-HDz⁻ solution. A time of 40 minutes, with occasional swirling, was allowed for the reaction to complete and the flasks were filled to the mark with aqueous 0.1 M NH_4OH. This complex was not initially part of this study, but is nevertheless reported to show that (p-COO⁻)-HDz⁻ reacts with large metal cations in a ratio of 1:1, as was the case for Pb.

Hg(*p*-COOH-HDz) solutions

CONTINIOUS VARIATION

6.   Ni(p-COOH-HDz)$_3$ & Ni(HDz)$_2$

The solution of 5.11×10^{-4} M (p-COO$^-$)-HDz$^-$ in 0.1 M aqueous KOH was reacted with a solution of 5.11×10^{-4} M of Ni^{II} from aqueous NiSO$_4\cdot$6H$_2$O (5.10×10^{-5} moles). To each of the twelve 50.0 mL volumetric flasks, 0.00, 0.50, 1.00, 1.50, 2.00, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, 9.00, and 10.00 mL of 5.09×10^{-4} M aqueous Ni^{II} was added and also 10.00, 9.50, 9.00, 8.50, 8.00, 7.50, 7.00, 6.00, 5.00, 4.00, 3.00, 2.00, 1.00 and 0.00 mL of 5.11×10^{-4} M (p-COO$^-$)-HDz$^-$ solution, respectively. A time of 40 minutes, with occasional swirling, was allowed for the reaction to complete and the flasks then filled to the mark with aqueous 0.1 M KOH.

5.11×10^{-4} M HDz$^-$ in 0.1 M aqueous NH$_4$OH reacted with aqueous of 5.06×10^{-4} M Ni^{II} from AgNO$_3$ (0.043 g, 2.53×10^{-4} moles). To each of the twelve 50.0 mL volumetric flasks, 0.00, 2.00, 4.00, 6.00, 8.00, 10.00, 12.00, 14.00, 16.00, 18.00, and 20.00 mL of 5.09×10^{-4} M aqueous Ni^{II} was added and also 20.00, 18.00, 16.00, 14.00, 12.00, 10.00, 8.00, 6.00, 4.00, 2.00, and 0.00 mL of 5.11×10^{-4} M (p-COO$^-$)-HDz$^-$ solution, respectively. A time of 40 minutes, with occasional swirling, was allowed for the reaction to complete and the flasks then filled to the mark with 0.1 M aqueous NH$_4$OH.

7.   Co(p-COOH-HDz)$_3$

The solution of 5.11×10^{-4} M (p-COO$^-$)-HDz$^-$ in 0.1 M aqueous NH$_4$OH was reacted with aqueous solutions of 5.09×10^{-4} M Co^{II} from aqueous CoCl$_2\cdot$6H$_2$O (5.09×10^{-5} moles). To each of the thirteen 50.0 mL volumetric flasks, 0.00, 0.50, 1.00, 1.50, 2.00, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, 9.00 and 10.00 mL of 5.09×10^{-4} M aqueous Co^{II} was added and also 10.00, 9.50, 9.00, 8.50, 8.00, 7.50, 7.00, 6.00, 5.00, 4.00, 3.00, 2.00, 1.00 and 0.00 mL of 5.11×10^{-4} M (p-COO$^-$)-HDz$^-$ solutions, respectively. A time of 40 minutes was allowed, with occasional swirling, for the reaction to complete and the flasks were filled to the mark with aqueous 0.1 M NH$_4$OH.

MOLE RATIO

Ag(p-COOH-HDz)

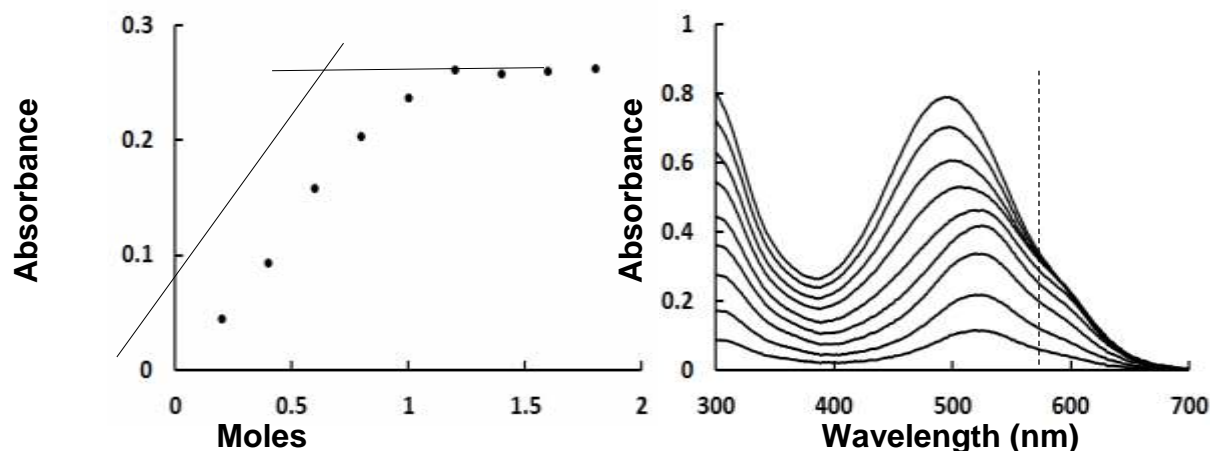**Figure SI-4.** Left: Plot of absorbance *vs* mole (*p*-COO⁻)-HDz⁻ ligand per mole **Ag^I** cation, in aqueous 0.1 M **NH$_4$OH**, at 590 nm. Right: Overlay spectra, dashed line corresponds to data points used in mole ratio plot.
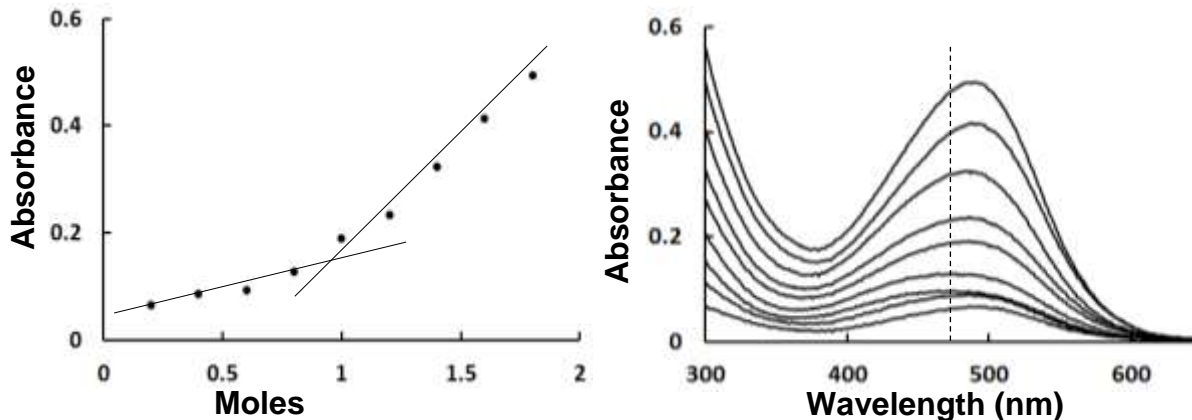
Pb(*p*-COOH-HDz)

Figure SI-5. Left: Plot of absorbance *vs* mole (*p*-COO⁻)-HDz⁻ ligand per mole **Pb^{II}** cation, in aqueous 0.1 M **NH$_4$OH**, at 490 nm. Right: Overlay spectra, dashed line corresponds to data points used in mole ratio plot.

The lead carboxylic acid dithizonate complex (1:1) is an unexpected different stoichiometry to the unsubstituted dithizonate complex (1:2). MS fails to confirm these results, neither being indicative of either 1:1, 1:2 or 1:3 complexes. This may possibly be ascribed to dissociation during the MS ionization process. The spectrophotometric tests may however be trusted by extrapolation simply because the coordination ratio's of other metal dithizonates (Ni, Co, Ag) are proven to be correct, also by other means of characterisation. All other metal dithizonates (Co, Ag) gave the same coordination number, i.e. Co(HDz)$_3$ and Co(*p*-COOH-HDz)$_3$ and also Ag(HDz) and Ag(*p*-COOH-HDz), with their ^{1}H NMR yielding the expected number of aromatic protons.

Ni(*p*-COOH-HDz)$_3$
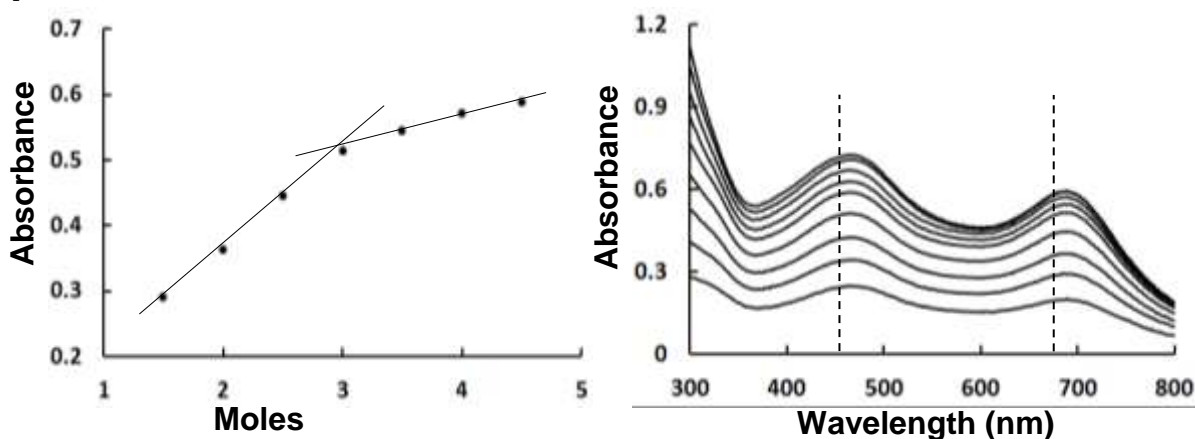
Figure SI-6. Left: Plot of absorbance *vs* mole (*p*-COO⁻)-HDz⁻ ligand per mole **Ni^{II}** cation, in aqueous 0.1 M **KOH** at 690 nm (similar trend observed at 465 nm). Right: Overlay spectra, dashed lines correspond to data points used in mole ratio plot.

Due to the unexpected 1:3 coordination ratio found for the Ni(II) complex, this experiment was also repeated in a polar **organic solvent**, namely acetone, and without the presence of base. The ligand, (*p*-COOH)-H_2Dz (0.0176 g, 5.11 x 10^{-5} moles) was dissolved in acetone in a 100.0 mL volumetric flask. An ultrasonic bath was used, obtaining the 5.11 x 10^{-4} M (*p*-COOH)-H_2Dz solution. $NiSO_4 \cdot 6H_2O$ (0.0134 g, 5.10 x 10^{-5} moles) was dissolved in warm methanol in a 100.0 mL volumetric flask, then filled up to the mark to get a solution of 5.10 x 10^{-4} M. To each of the seven 50.0 mL volumetric flasks, 2.00 mL of 5.09 x 10^{-4} M methanolic Ni^{II} solution was added and also 1.00, 2.00, 3.00, 4.00, 5.00, 6.00 and 7.00 mL of 5.11 x 10^{-4} M *p*-COOH-H_2Dz solution respectively. A time period of 40 minutes was allowed, with occasional swirling, for the reaction to complete, and the flasks were then filled to the mark with acetone. In the absence of base (KOH or NH_4OH) the nickel complex now yielded a 1:2 ratio, Ni(*p*-COOH-HDz)$_2$, see Figure 3.29. Here, the COOH substituents are not deprotonated and thus not readily available for complexation. Typically here the Ni complex has three absorption peaks (465, 560 and 690 nm). Although the slopes of the two straight lines giving the combining ratio differ not much, it is nevertheless seen that the ratio of the Ni^{II} cation to (*p*-COO$^-$)-HDz$^-$ ligand is 1:2. Mass spectroscopy results indicate a third (*p*-COO$^-$)-HDz$^-$ ligand coordinated to Ni, most probably via a –COO$^-$ phenyl substituent.

In using the unsubstituted **H_2Dz**, a 1:2 ratio, Ni(HDz)$_2$, was oberved in both bases, KOH and NH_4OH. The role of the carboxylate anion is therefore evendent in coordination ratio's involving nickel. The overlay spectra in Figure 3.30 has λ_{max} = 420 and 660 nm for the Ni(HDz)$_2$ complex. Repeatable results were only obtained at 420 and 510 nm, while absorbance data taken at 660 nm were not consistent. Also when using 0.2 M instead of 0.1 M aqueous NH_4OH, the exact same spectra were observed, with the orange colour not changing; the intensity merely increases in a non-linear fashion with increase in HDz$^-$ concentration.
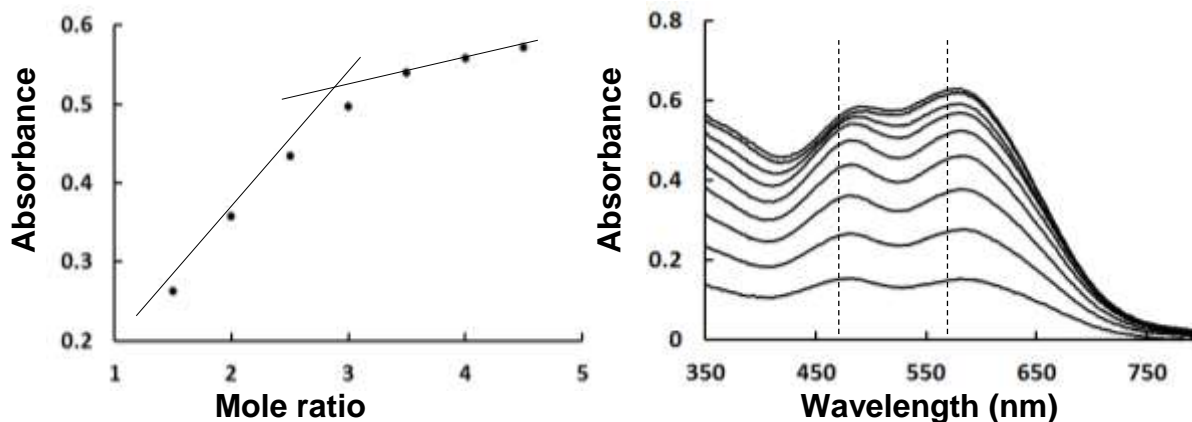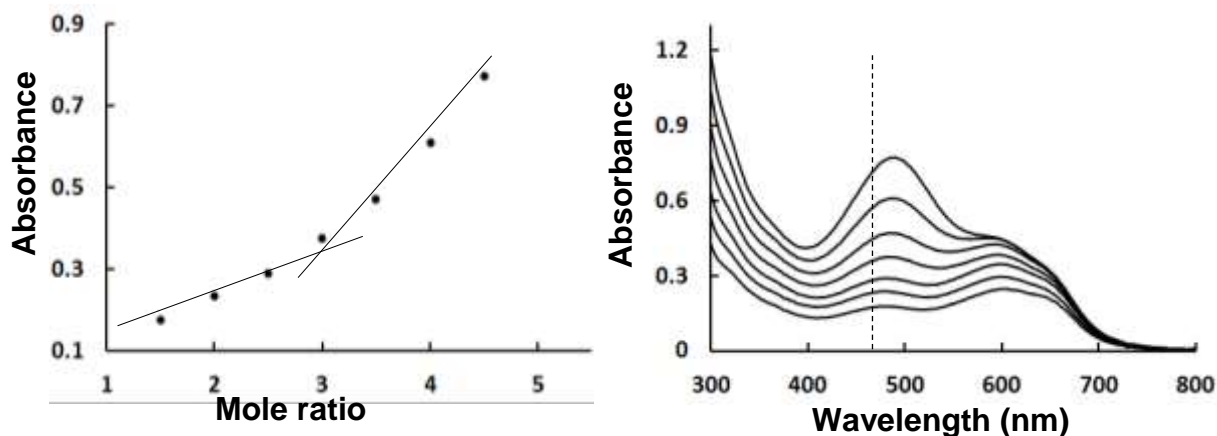
Co(*p*-COOH-HDz)$_3$

Figure SI-7. Left: Plot of absorbance *vs* mole (***p*-COO$^-$)-HDz$^-$** ligand per mole **Co^{II}** cation, in aqueous 0.1 M **KOH** at 490 nm (similar trend observed at 580 nm). Right: Overlay spectra, dashed lines correspond to data points used in mole ratio plot.

Figure SI-8. Left: Plot of absorbance *vs* mole (*p*-COO⁻)-HDz⁻ ligand per mole Co^{II} cation, in aqueous 0.1 M NH₄OH at 490 nm. Right: Overlay spectra, dashed line corresponds to data points used in mole ratio plot.

Results shown in Figures 3.25 and 3.26 are indicative of the Co^{II} cation reacting in a ratio of 1:3 with (*p*-COO⁻)-HDz⁻. These results are not affected by the type of base, namely NH₄OH or KOH.
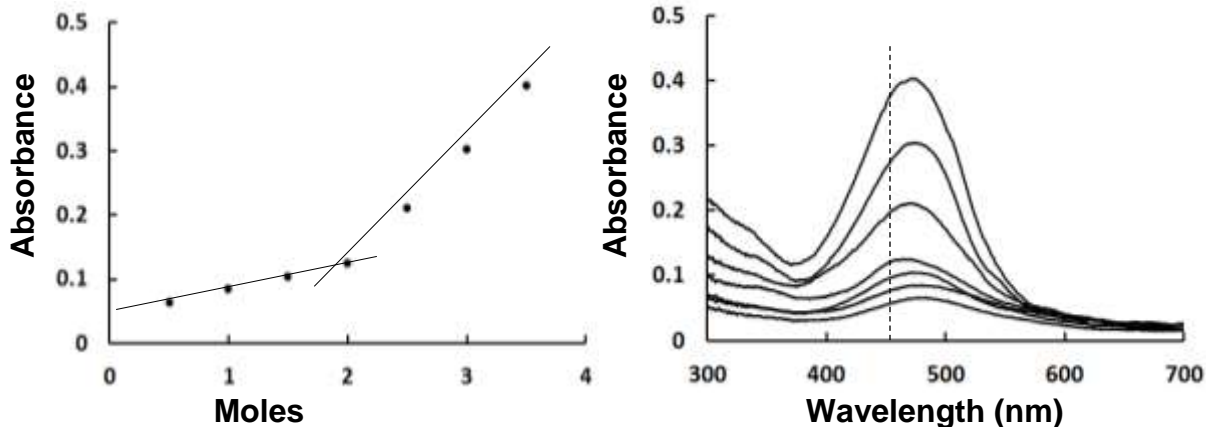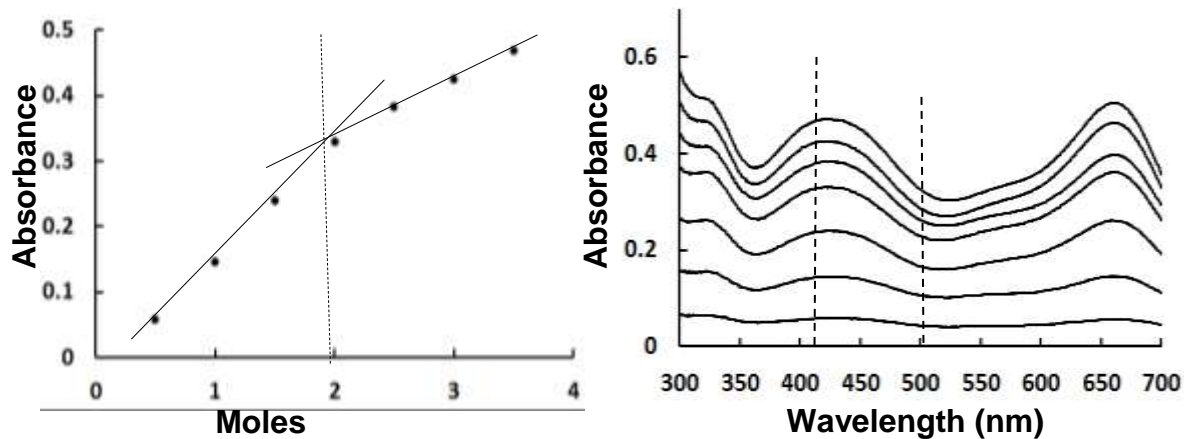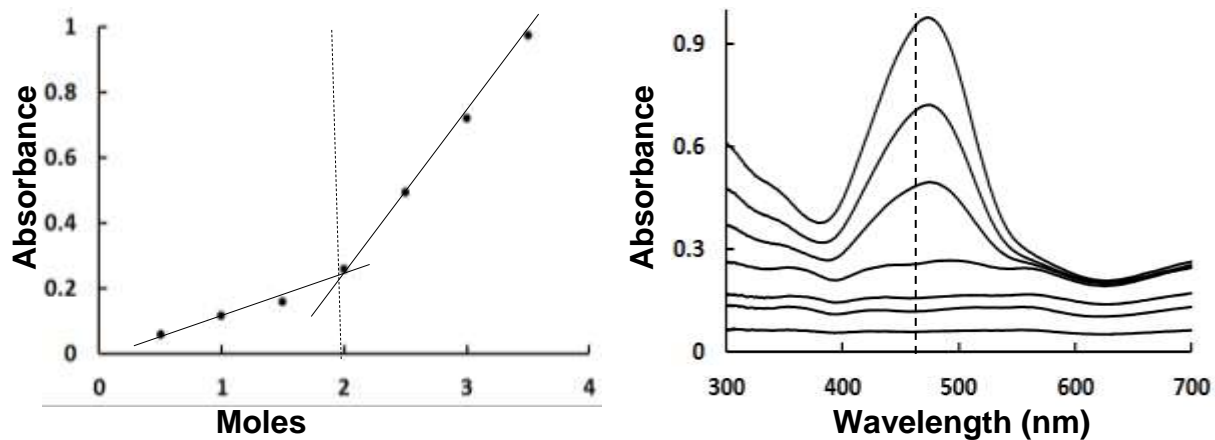
Ag(HDz)
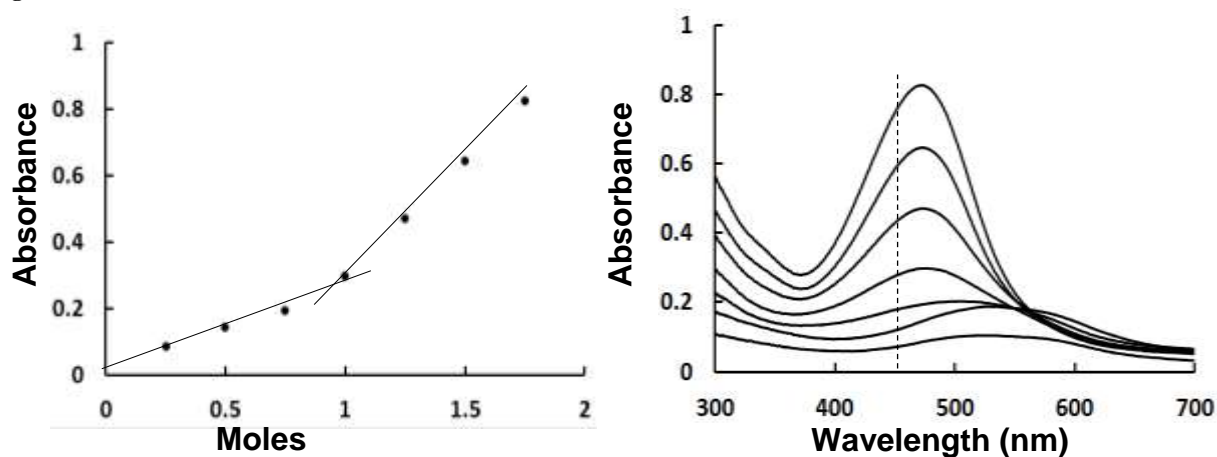
Figure SI-9. Left: Plot of absorbance *vs* mole **HDz⁻** ligand per mole **Ag^{I}** cation, in aqueous 0.1 M **KOH** at 470 nm. Right: Overlay spectra, dashed line corresponds to data points used in mole ratio plot.

Pb(HDz)₂

Figure SI-10. Left: Plot of absorbance *vs* mole **HDz⁻** ligand per mole **Pbᴵᴵ** cation, in aqueous 0.1 M **NH₄OH** at 470 nm. Right: Overlay spectra, dashed line corresponds to data points used in mole ratio plot.
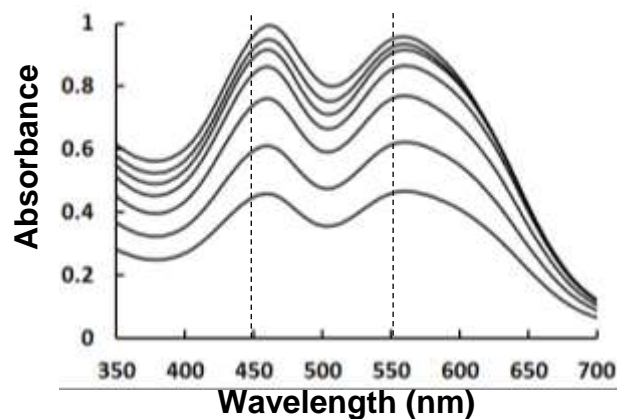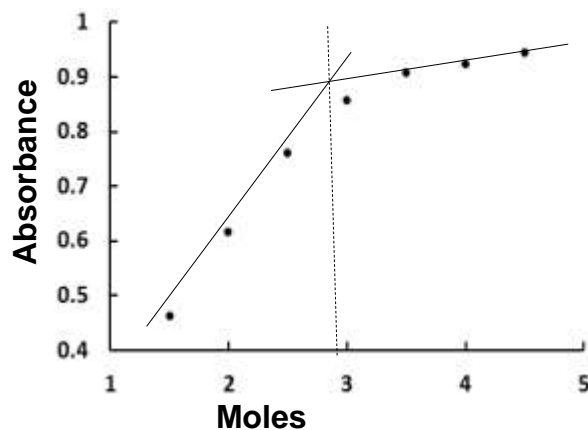
Ni(HDz)₂

Figure SI-11. Left: Plot of absorbance *vs* mole **HDz⁻** ligand per mole **Niᴵᴵ** cation, in aqueous 0.1 M **KOH** at 420 nm (similar trend observed at 510 nm). Right: Overlay spectra, dashed lines correspond to data points used in mole ratio plot.

Figure SI-12. Left: Plot of absorbance *vs* mole **HDz⁻** ligand per mole **Niᴵᴵ** cation, in aqueous 0.1 M **NH₄OH** at 470 nm. Right: Overlay spectra, dashed line corresponds to data points used in mole ratio plot.

Co(HDz)₃

Figure SI-13. Left: Plot of absorbance *vs* mole **HDz⁻** ligand per mole **Coᴵᴵ** cation, in aqueous 0.1 M **KOH** at 570 nm (similar trend observed at 470 nm). Right: Overlay spectra, dashed lines correspond to data points used in mole ratio plot.
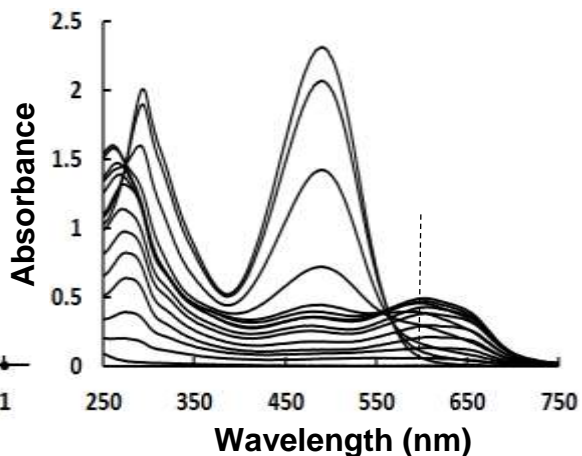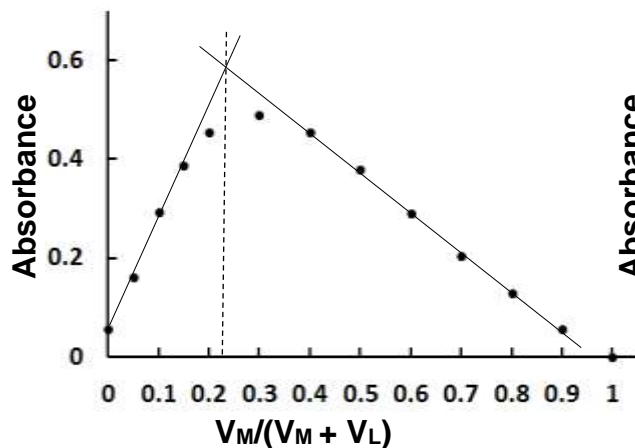
CONTINUOUS VARIATION

Co(*p*-COOH-HDz)₃

Figure SI-14**.** Left: Plot of corrected absorbance *vs* (*p*-**COO⁻**)-**HDz⁻** volume fraction of **Coᴵᴵ** cation, in 0.1 M **NH₄OH**, at 600 nm. Right: Overlay spectra, dashed line corresponds to data points used in continuous variation plot.
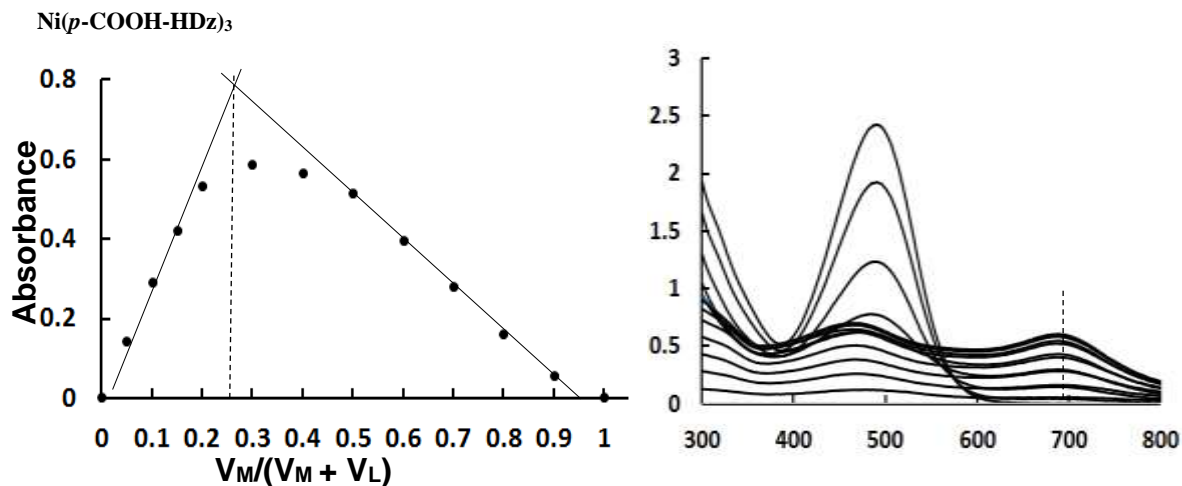
Ni(*p*-COOH-HDz)₃

Figure SI-15. Left: Plot of corrected absorbance *vs* (*p*-COO⁻)-HDz⁻ volume fraction of **Ni^{II}** cation, in aqueous 0.1 M **KOH**, at 700 nm. Right: Overlay spectra, dashed line corresponds to data points used in mole ratio plot.
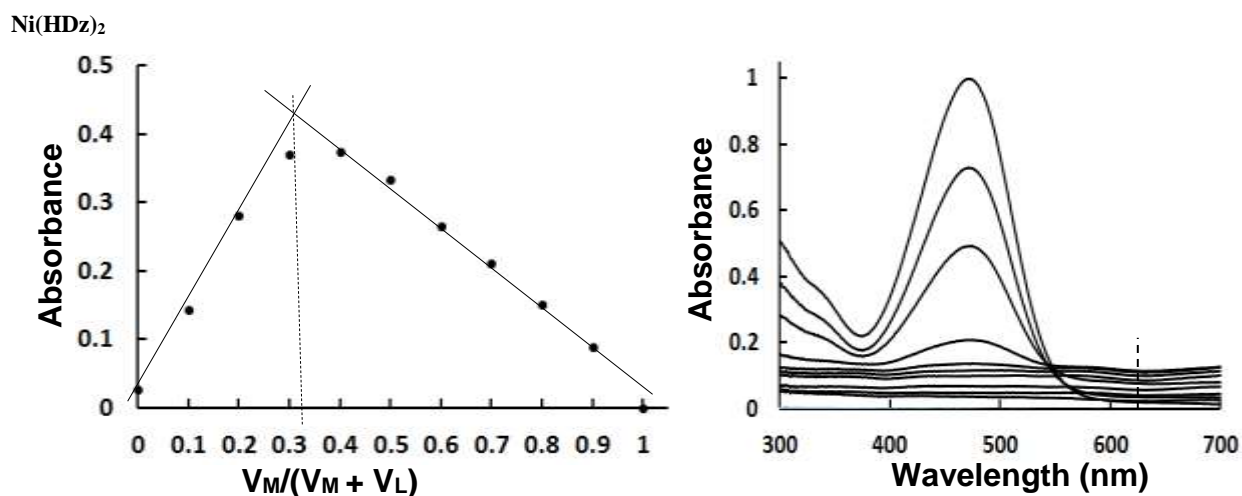
Ni(HDz)₂

Figure SI-16. Left: Plot of corrected absorbance *vs* mole **HDz⁻** volume fraction of **Ni^{II}** cation, in aqueous 0.1 M **NH₄OH** at 630 nm with the dashed line at maximum volume ratio. Right: Overlay spectra, dashed line corresponds to data points used in mole ratio plot.

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