

Solution equilibria studies of complexes of divalent metal ions with 2-aminophenol and 3,4-dihydroxybenzoic acid



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ARTICLE INFO

Article history:

Received 1 August 2014

Accepted 28 November 2014

Available online 27 December 2014

Keywords:

3,4-Dihydroxybenzoic acid

2-Aminophenol

pH-potentiometry

Hyperquad2008

UV-Vis spectrophotometry

ABSTRACT

The chelation abilities of 2-aminophenol and 3,4-dihydroxybenzoic acid with divalent metal ions (Cu^{2+} , Be^{2+} , Zn^{2+} , Ni^{2+} , Co^{2+} and Mn^{2+}) in binary and ternary systems at 37 ± 0.1 °C and an ionic strength of 0.15 mol dm^{-3} NaCl were systematically investigated by using the potentiometric titration method. The chelating abilities of these complexes were obtained by processing the titration data using the Hyperquad2008 program and the results are presented as stability constants. In a binary system, it was shown that metal complexation involving 3,4-dihydroxybenzoic acid (ligand D) is more stable than the one with 2-aminophenol (ligand A). The stability of the formed metal complexes, both in binary and ternary systems, decreases in the following order: $\text{Cu}^{2+} > \text{Be}^{2+} > \text{Zn}^{2+} > \text{Ni}^{2+} > \text{Co}^{2+} > \text{Mn}^{2+}$. The tendency of these metals and ligands to form binary or ternary complexes was also evaluated and discussed by calculating their $\Delta \log K_M$ and $\log X$ values. In addition, the distribution of complex species in these systems was graphically presented using the HySS2009 program. UV-Vis spectrophotometry was also performed to qualitatively verify the protonation of these ligands and to confirm the model of the complex formed.

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1. Introduction

The earth is rich in various metals which are distinguished by their electronic distributions, that in turn affect their physiological and pathological characteristics. Several metals, which are part of dietary minerals, are found in every living organism and they take part in the functionality of body systems; in particular as stabilizers of biological structures, components of control mechanism (in nerves and muscles) and activators or components of redox systems [1,2]. In other words, some metals are categorized as essential elements, meaning the lack of them will affect the biological functions of the human body. In contrast, their presence in excess levels is toxic and may have adverse effects on human health.

Chelation therapy has been known as one of the medical treatments that is able to reduce excess metal levels in the human body and indirectly prevent carcinogenesis and cancer development. This therapy has been applied historically and it commonly employs certain chelating agents to reduce the body's burden

towards metal toxicity [3–5]. However, commercial chelators still bring some adverse effects and it sometimes leads to death [1,6–9]. Several new potential chelators, including vitamin, phenolic compounds and amino acids, are found to increase metal mobilization, which means they could minimize the possibility of the interaction of the metal ions with important bio-substances [10–12]. Several studies have reported that combining a chelating agent and an antioxidant may be beneficial for metal poisoning antidote therapy, by protecting the human body from reactive oxygen species and oxidative stress [1,12,13].

In this study, two phenolic compounds (2-aminophenol and 3,4-dihydroxybenzoic acid) were investigated for their potential as chelating agents. Both phenolics are known to possess different binding sites that may play important roles in their chelating activity. 2-Aminophenol contains neighboring N and O donor atoms in the aromatic ring, which allow the compound to form stable five-membered-ring complexes with metal ions [14]. The compound was also found to have antibacterial activity against gram-negative *Bacillus* [15,16]. Its administration into animals also showed no toxic effects and the compound was excreted in conjugation with both sulfuric acid and glucuronic acid [17]. Another potential chelator, 3,4-dihydroxybenzoic acid, was reported to have chemoprevention activity against carcinogenesis [18–20]. This compound

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was found to be effective in inhibiting colon, skin and liver cancers, either in the initiation phase or in the promotion/progression of carcinogenesis. It also shows antioxidant activity due to the occurrence of a carboxylic group and two adjacent hydroxyl groups. These functional groups may act as inhibitors of free radical reactions by forming complexes with transition metal ions (Cu^{2+} and Fe^{2+}) and thus may lower the activity of enzymes that catalyze the reactions of free radical formation [21,22].

In order to gain an initial understanding and basic information on metal chelation for further research in chelation therapy, this study investigated the complexation of 2-aminophenol and 3,4-dihydroxybenzoic acid with divalent metal ions (Mn^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} and Be^{2+}) in binary and ternary systems. While many stability constants of metal ions and 3,4-dihydroxybenzoic acid have been reported before, only very limited information can be found regarding the metal complexing abilities of the 2-aminophenol ligand. In addition, the usefulness of these chelating agents to reduce excess metal ions in ternary system with both ligands has never been investigated before. Therefore, it is necessary to understand the possible chelation processes and stable complexes formed between these chelators and certain metal ions. While most reported works were carried out at 25 °C and with an ionic strength 0–0.2 mol dm⁻³ NaCl, all the experimental studies in this work were carried out at physiological conditions which mimic body fluids (37 ± 0.1 °C and 0.15 mol dm⁻³ NaCl) to gain reliable results for further research in drug development for chelation therapy. The stability constant study helps to understand the interaction strength between the chelating agent and metal ion in forming complexes. The complexing abilities were investigated using a potentiometric method and the Hyperquad2008 program. The speciation model was determined using the HySS2009 program. UV–Vis spectrophotometry was performed to determine the first protonation constant of the 3,4-dihydroxybenzoic acid, to qualitatively verify the protonation of the ligands and to confirm the model of the complexes formed.

2. Materials and methods

2.1. Materials

The chemicals used in this study and their suppliers are as follows: 2-aminophenol (MW = 109.1; 0.99 purity) and 3,4-dihydroxybenzoic acid (MW = 154.1, 0.97 purity), Alfa Aesar (Lancashire, UK); nickel chloride hexahydrate (MW = 237.7, 0.98 purity) and beryllium sulfate tetrahydrate (MW = 177.1, 0.98 purity), Alfa Aesar (Lancashire, UK); zinc nitrate hexahydrate (MW = 297.5, 0.98 purity) and manganese chloride tetrahydrate (MW = 197.9, 0.998 purity), Acros Organics (Morris Plains, NJ); cobalt nitrate hexahydrate (MW = 291.0, 0.98 purity), copper chloride dihydrate (MW = 170.5, 0.99 purity), potassium hydrogen phthalate (MW = 58.45, 0.99 purity), Sigma Aldrich (Steinheim, Germany); sodium hydroxide (MW = 40, 0.96 purity), Yakuri Pure Chemicals (Kyoto, Japan); sodium chloride (MW = 58.44, 0.995 purity), Showa (Tokyo, Japan); hydrochloric acid (MW = 63.01, 0.376 purity), Fisher Scientific (Hampton, NH). All chemicals were directly used without further purification. Solutions were prepared daily in distilled deionized water (resistance >18 M Ω). In particular, solutions of metal salts, NaOH and HCl were standardized before used.

2.2. Potentiometric measurement

The following solutions, each with a total volume of 50 cm³, were prepared in a 100 cm³ double walled equilibrium cell for

pH-potentiometric measurements and each was titrated against standard carbonate-free NaOH (0.10 mol dm⁻³).

- 3×10^{-3} mol dm⁻³ HCl + 1.5×10^{-1} mol dm⁻³ NaCl.
- Solution a + 1×10^{-3} mol dm⁻³ 2-aminophenol (A) or 3,4-dihydroxybenzoic acid (D).
- Solution a + 1×10^{-3} – 1.2×10^{-3} mol dm⁻³ A or D + 4×10^{-4} – 1×10^{-3} mol dm⁻³ metal salt.
- Solution a + 5×10^{-4} – 1×10^{-3} mol dm⁻³ A + 4×10^{-4} – 1×10^{-3} mol dm⁻³ D + 4×10^{-4} – 1×10^{-3} mol dm⁻³ metal salt.

Prior to titration, each solution was kept at 37 ± 0.1 °C for 15 min by connecting the equilibrium cell to a refrigerated circulating bath equipped with a thermocouple to closely monitor the reaction temperature. During potentiometric measurements, the temperature and ionic strength of the system were maintained at a constant 37 ± 0.1 °C and 0.15 mol dm⁻³ NaCl, respectively. At least three repetitions for the pH-potentiometry procedure were carried out for each experiment, with a reproducibility of ±0.02 in pH units.

The potentiometric titrations were performed using a meter-ohm 888-Titrando Autotitrator equipped with a Dosimat model 805, 802 rod stirrer and 804 Ti stand, and an Ecotrode Plus pH glass electrode was used for potentiometric measurements. The instrument was connected to a personal computer equipped with Tiamo 2.3 computer software to control the titration conditions and record the experimental data. The glass electrode response was able to give a pH reading with a precision of ±0.001. Prior to use, the electrode was calibrated with standard buffer solutions (pH 4.00, 7.00 and 10.00) at the same temperature used for the main experimental work (37 ± 0.1 °C). Since the determination of metal–ligand stability constants was carried out at high ionic strength (0.15 mol dm⁻³ NaCl), it was necessary to carry out a strong acid–strong base titration for the glass electrode calibration. For this purpose, the titration curve of solution a was processed using the Glass Electrode Evaluation (GLEE) program to evaluate the calibration of the glass electrode and to estimate carbonate contaminant for each investigation [23].

The titration curve of solution b was later processed by the Hyperquad2008 program in order to determine the protonation constant of the ligands. In contrast, the investigations of metal ligand stability constants in the binary and ternary systems were carried out by processing the titration curves of solutions c and d, respectively. As shown in Table 1, the metal ligand complexation studies in the binary system were done at four different metal-to-ligand ratios, while ternary studies were done at three different metal:ligand A:ligand D ratios, to cover all possible complex species that may occur in the system.

Table 1
Concentrations of metal and ligand in solution c that were used to determine the overall formation constants of binary complexes.

Ratios	Concentration (mol dm ⁻³)		
	Metal	Ligand A	Ligand D
<i>Binary [M]:[L]</i>			
1:1	1.0×10^{-3}	1.0×10^{-3}	
1:2	5.0×10^{-4}	1.0×10^{-3}	
1:2.5	4.0×10^{-4}	1.0×10^{-3}	
1:3	4.0×10^{-4}	1.2×10^{-3}	
<i>Ternary [M]:[A]:[D]</i>			
1:1:1	1.0×10^{-3}	1.0×10^{-3}	1.0×10^{-3}
1:1:2	5.0×10^{-4}	5.0×10^{-4}	1.0×10^{-3}
1:2:1	5.0×10^{-4}	1.0×10^{-3}	5.0×10^{-4}

2.3. Software analysis

Hyperquad2008 was used to determine the formation constants of the complex species formed in the system using a non-linear least-squares algorithm. Various models with possible compositions of complex species were proposed in the program and the one that gave the best fitted model was chosen [24,25]. The goodness of fitting was statistically presented as a sigma (σ) value and was obtained from the following equation:

$$\sigma = \sqrt{\frac{\sum_{i=1, np} (W_i r_i^2)}{(m - n)}} \quad (1)$$

where W_i , r_i , m and n refer to the weight at the i th data point, the residual at the i th data point, the number of titration data points and the refined parameters, respectively. In this study, the number

Table 2
Protonation constants^a ($\log K_n$) of ligands A and D at 37 ± 0.1 °C, $I = 0.15$ mol dm⁻³ NaCl.

Ligand	$\log K_1 \pm \text{SD}^b$	$\log K_2 \pm \text{SD}^b$	$\log K_3 \pm \text{SD}^b$	σ
A	9.58 ± 0.01 (9.87 ^c)	4.53 ± 0.01 (4.74 ^c)		1.22
D	12.75 ± 0.02 (12.80 ^d ; 13.10 ^e)	8.62 ± 0.01 (8.70 ^d ; 8.67 ^e)	4.42 ± 0.01 (4.50 ^d ; 4.25 ^e)	1.17

^a All measurements were carried out by potentiometric measurements and with the Hyperquad software, except for the $\log K_1$ value of ligand D, which was investigated by UV–Vis spectrophotometry measurements and the HySpec software.

^b Standard deviation value of the last decimal place; $\pm 95\%$ confidence interval.

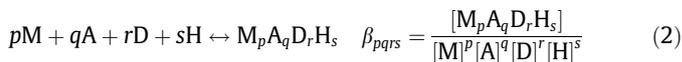
^c Ref. [33], $T = 25$ °C; $I = 0.1$ mol dm⁻³ KCl.

^d Ref. [30], $T = 25$ °C; $I = 0.02$ mol dm⁻³ KNO₃.

^e Ref. [35], $T = 25$ °C; $I = 0.2$ mol dm⁻³ KCl.

of points used for each refinement was between 80 and 200 points. The residual at the i th data point is defined as the difference between the observed and the calculated pH values. The fitting model is considered to be good if the sigma (σ) value is in the range 1.17–1.35. It specifically represents 95% goodness of fitting [24].

The result of refinement is generally expressed as the overall formation constant (β_{pqrs}) which is described in Eq. (2):



where the variables p , q , r and s represent the stoichiometric coefficients of the metal ions (M), 2-aminophenol (A), 3,4-dihydroxybenzoic acid (D) and hydrogen ions (H), respectively. A higher value of this constant indicates greater stability of the complex [26,27]. All standard deviations are provided directly from refinement of the Hyperquad software. In order to support the result from the Hyperquad2008 program, HySS2009 was used to simulate and illustrate the possible species distribution of ligand protonation and metal–ligand complex at different pH values [28].

2.4. UV–Vis spectrophotometric measurement

All spectrophotometric measurements were carried out in standard 1 cm quartz cells at 200–800 nm by using a JASCO V-550 spectrophotometer. Prior to the measurements, the following solutions were prepared with a total volume of 50 cm³:

- 1.5×10^{-1} mol dm⁻³ NaCl + 2×10^{-4} mol dm⁻³ ligand D.
- 1.5×10^{-1} mol dm⁻³ NaCl + 2×10^{-4} mol dm⁻³ ligand A or D + 1×10^{-4} mol dm⁻³ metal salt.
- 1.5×10^{-1} mol dm⁻³ NaCl + 2×10^{-4} mol dm⁻³ ligand D + 2×10^{-4} mol dm⁻³ metal salt.

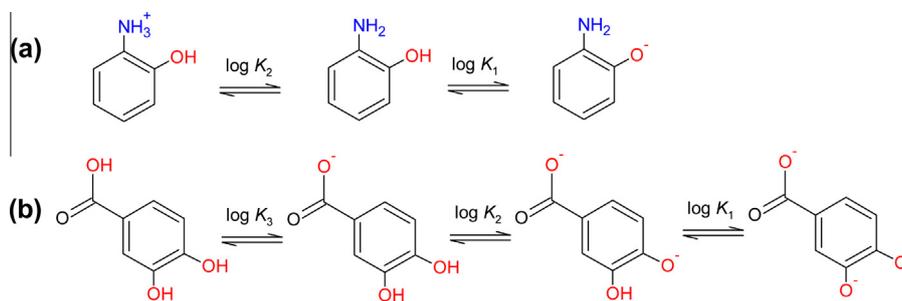


Fig. 1. Protonation equilibria for: (a) 2-aminophenol (A); and (b) 3,4-dihydroxybenzoic acid (D).

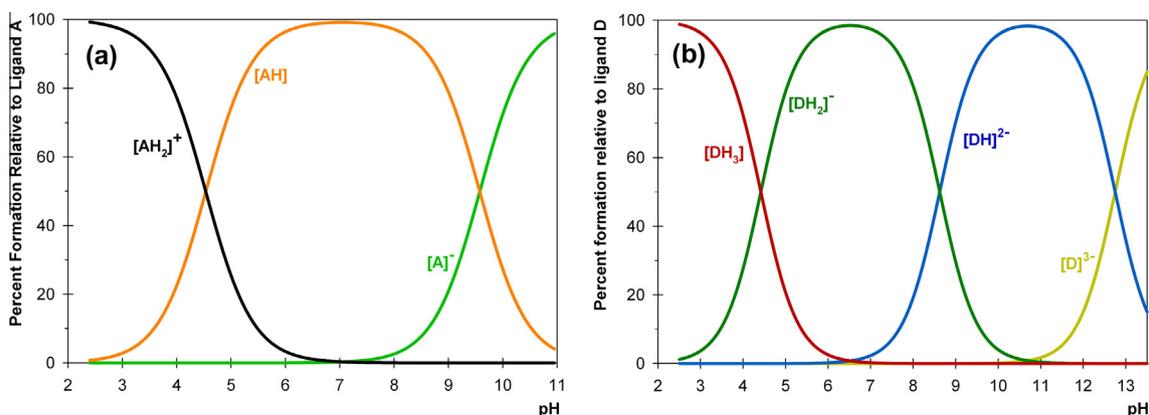


Fig. 2. Species distribution acid disassociation of a solution containing 0.001 mol dm⁻³ of (a) 2-aminophenol (A); (b) 3,4-dihydroxybenzoic acid (D) at 37 ± 0.1 °C and $I = 0.15$ mol dm⁻³ NaCl.

(iv) $5 \times 10^{-2} \text{ mol dm}^{-3}$ ligand A + $5 \times 10^{-2} \text{ mol dm}^{-3}$ ligand D + $5 \times 10^{-2} \text{ mol dm}^{-3}$ metal salt.

The temperatures of these solutions were maintained at $37 \pm 0.1 \text{ }^\circ\text{C}$. The spectra of solution (i) between pH 2.5 and 13.5 were used to determine the protonation constant of ligand D. While solutions (ii) and (iii) were used to determine the stability constants of the complexes in the binary and ternary systems, respectively. The values will be used later to support the stability constants obtained from potentiometric measurements. The UV–Vis spectra of solutions (i)–(iii) at pH 2.5 to 11.0 then were processed in the Hypspec2006 program to refine the constants [28]. Further confirmation of the *d–d* block transition spectrum of the complexes was performed on the ternary system. For this purpose, the spectra of solution (iv) were scanned at pH 2.5 and 11.0. Three repetitions were done for each measurement and each system was maintained with conditions similar to that used in the potentiometric titration. NaOH was used to adjust the system pH to the desired value.

3. Results and discussion

3.1. Protonation constants

Protonation constants are used in metal chelation studies to predict at which pH the ligand is ionized and able to form complexes with metal ions. In this work, the protonation of 2-aminophenol (ligand A) and 3,4-dihydroxybenzoic acid (ligand D) was studied by using a potentiometric titration method and their protonation constants, expressed as stepwise association constants ($\log K$), were determined using the Hyperquad2008 program. As presented in Table 2, the $\log K$ values of ligands A and D obtained in this study are comparable to those reported in the literature, after taking into consideration the differences in experimental conditions [29,30].

Ligand A possesses two functional groups, the first protonation occurred at $\log K = 9.58$, followed by a second protonation at $\log K = 4.53$. The amino group ($-\text{NH}_2$) is known to be more acidic than the phenol group ($-\text{OH}$), thus it was suggested that the first

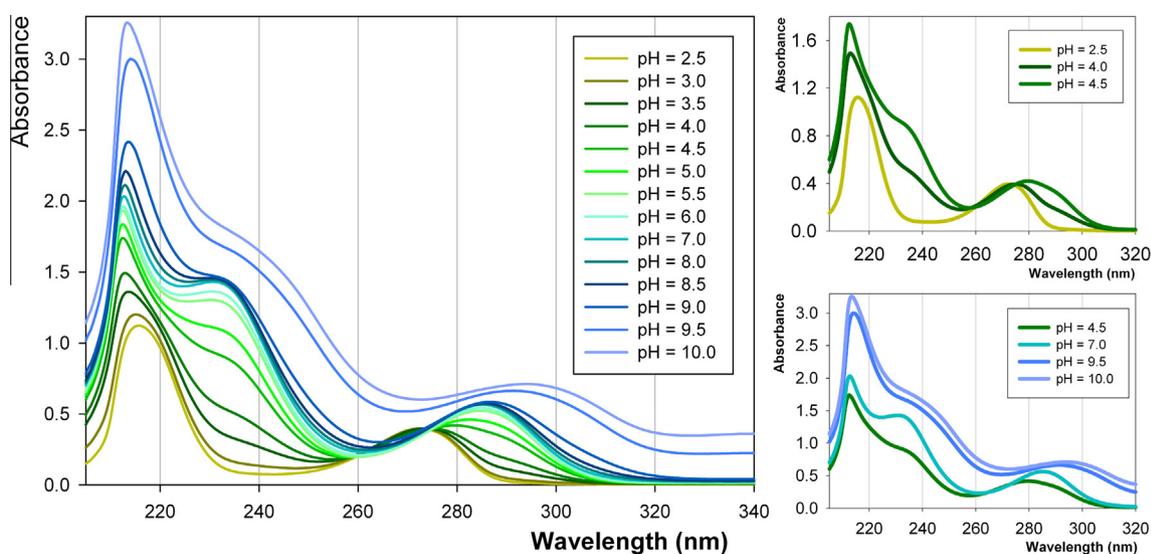


Fig. 3. UV–Vis spectra of 2-aminophenol (A), where $[A] = 0.001 \text{ mol dm}^{-3}$, $T = 37 \pm 0.1 \text{ }^\circ\text{C}$, $I = 0.15 \text{ mol dm}^{-3} \text{ NaCl}$.

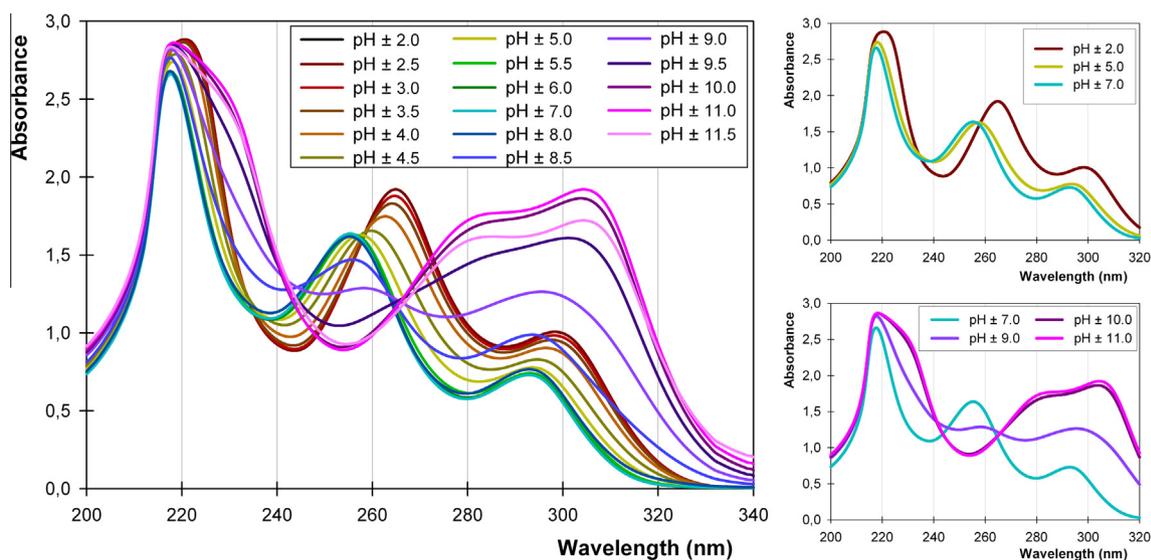


Fig. 4. UV–Vis spectra of 3,4-dihydroxybenzoic acid (D), where $[D] = 0.001 \text{ mol dm}^{-3}$, $T = 37 \pm 0.1 \text{ }^\circ\text{C}$, $I = 0.15 \text{ mol dm}^{-3} \text{ NaCl}$.

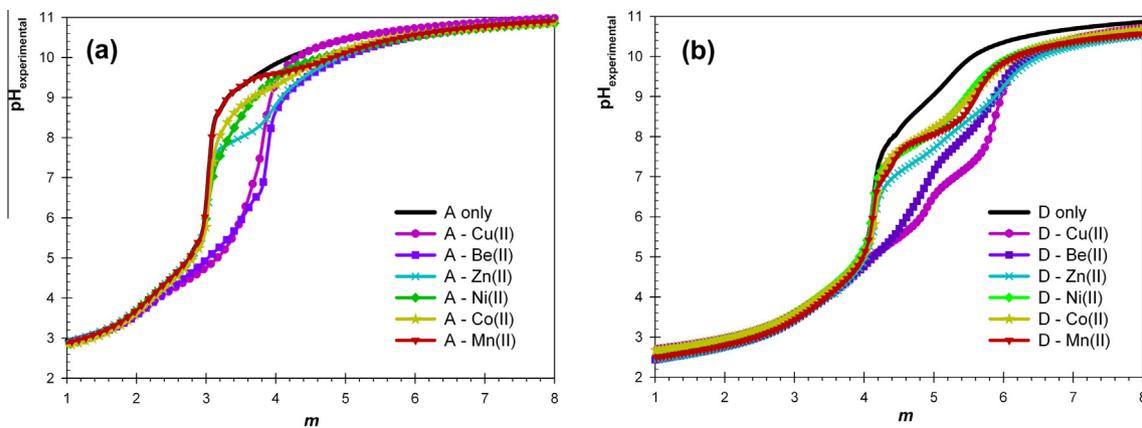


Fig. 5. Experimental curves of the binary system containing: (a) 2-aminophenol (A), (b) 3,4-dihydroxybenzoic acid (D) and divalent metal ions, where m is the moles of base added per mole of ligand.

Table 3

Logarithms of overall stability constants of 2-aminophenol (A) with several metals ($\log \beta_{pqrs}$) at 37 ± 0.1 °C, $I = 0.15$ mol dm⁻³ NaCl.

Complex species	p	q	s	$\log \beta_{pqrs}^a \pm SD^b$		Ref.
				Hyperquad	Hypspec	
$M = Cu^{2+}$						
[MAH] ²⁺	1	1	1	12.61 ± 0.04	–	–
[MA] ⁺	1	1	0	8.48 ± 0.01	8.61 ± 0.01	8.36 ^c
[MA ₂]	1	2	0	14.84 ± 0.04	14.94 ± 0.08	–
($\sigma = 1.22$) ^d						
$M = Be^{2+}$						
[MAH] ²⁺	1	1	1	13.08 ± 0.02	14.03 ± 0.03	–
[MA] ⁺	1	1	0	8.12 ± 0.01	8.04 ± 0.03	–
[MA ₂]	1	2	0	14.86 ± 0.03	14.47 ± 0.08	–
[MA ₃] ⁻	1	3	0	19.62 ± 0.06	–	–
($\sigma = 1.29$) ^d						
$M = Zn^{2+}$						
[MA] ⁺	1	1	0	5.26 ± 0.01	5.18 ± 0.02	–
[MA ₂]	1	2	0	9.72 ± 0.04	10.07 ± 0.08	–
($\sigma = 1.33$) ^d						
$M = Ni^{2+}$						
[MA] ⁺	1	1	0	4.74 ± 0.01	4.76 ± 0.02	–
[MA ₂]	1	2	0	8.57 ± 0.02	8.66 ± 0.03	–
($\sigma = 1.26$) ^d						
$M = Co^{2+}$						
[MA] ⁺	1	1	0	4.08 ± 0.02	4.27 ± 0.03	–
[MA ₂]	1	2	0	7.63 ± 0.03	7.96 ± 0.04	–
($\sigma = 1.33$) ^d						
$M = Mn^{2+}$						
[MA] ⁺	1	1	0	3.52 ± 0.11	3.36 ± 0.05	–
[MA ₂]	1	2	0	6.64 ± 0.07	6.91 ± 0.04	–
($\sigma = 1.30$) ^d						

^a p , q , and s represent the stoichiometric coefficients of the metal ions, 2-aminophenol (A) and the proton of the ligand, respectively.

^b Standard deviation value of the last decimal place; $\pm 95\%$ confidence interval.

^c Ref. [35], $T = 25$ °C; $I = 0.2$ mol dm⁻³ NaClO₄.

^d The sigma (σ) value expresses the goodness of potentiometric data fitting using Hyperquad2008.

protonation of ligand A occurred at its phenol site [31]. While for ligand D, due to its vicinal diol and carboxylic functional groups, this ligand has been shown to have three protonation constants ($\log K_1 = 12.75$, $\log K_2 = 8.62$ and $\log K_3 = 4.42$). Deprotonation of ligand D starts at its carboxyl group, followed by second and third acid deprotonations at its less acidic para and meta hydroxyl groups, respectively [32]. The hydroxyl substitute in the para position is much easier to deprotonate due to a resonance effect [33]. This effect is particularly influenced by the occurrence of the negative-charged carboxylic group, which results in electron delocal-

Table 4

Logarithms of overall stability constants of 3,4-dihydroxybenzoic acid (D) with several metals ($\log \beta_{pqrs}$) at 37 ± 0.1 °C, $I = 0.15$ mol dm⁻³ NaCl.

Complex species	p	r	s	$\log \beta_{pqrs}^a \pm SD$		Ref.
				Hyperquad	Hypspec	
$M = Cu^{2+}$						
[MDH ₂] ⁺	1	1	2	23.94 ± 0.02	24.99 ± 0.09	–
[MDH]	1	1	1	19.09 ± 0.01	19.31 ± 0.07	19.22 ^b
[MD] ⁻	1	1	0	13.77 ± 0.01	13.92 ± 0.04	13.95 ^b
[MD ₂] ⁴⁻	1	2	0	24.49 ± 0.01	–	25.02 ^b
($\sigma = 1.21$) ^c						
$M = Be^{2+}$						
[MDH]	1	1	1	19.60 ± 0.01	19.91 ± 0.03	–
[MD] ⁻	1	1	0	13.52 ± 0.01	13.74 ± 0.04	–
[MD ₂] ⁴⁻	1	2	0	22.71 ± 0.02	22.47 ± 0.06	–
[MD ₃] ⁷⁻	1	3	0	28.12 ± 0.06	–	–
($\sigma = 1.21$) ^c						
$M = Zn^{2+}$						
[MDH]	1	1	1	–	18.00 ± 0.05	–
[MD] ⁻	1	1	0	8.71 ± 0.01	8.65 ± 0.02	8.91 ^c
[MD ₂] ⁴⁻	1	2	0	15.56 ± 0.01	15.27 ± 0.04	15.62 ^c
[MD ₃] ⁷⁻	1	3	0	21.93 ± 0.02	21.88 ± 0.09	–
($\sigma = 1.28$) ^c						
$M = Ni^{2+}$						
[MD] ⁻	1	1	0	8.06 ± 0.01	8.08 ± 0.07	8.27 ^c
[MD ₂] ⁴⁻	1	2	0	12.65 ± 0.05	–	12.98 ^c
[MD ₃] ⁷⁻	1	3	0	17.05 ± 0.09	–	–
($\sigma = 1.19$) ^c						
$M = Co^{2+}$						
[MD] ⁻	1	1	0	7.76 ± 0.01	7.85 ± 0.03	7.96 ^c
[MD ₂] ⁴⁻	1	2	0	12.52 ± 0.02	–	13.36 ^c
($\sigma = 1.30$) ^c						
$M = Mn^{2+}$						
[MD] ⁻	1	1	0	7.36 ± 0.02	7.52 ± 0.04	7.43 ^d
[MD ₂] ⁴⁻	1	2	0	12.52 ± 0.04	–	12.64 ^d
($\sigma = 1.21$) ^c						

^a p , q , and s represent the stoichiometric coefficients of the metal ions, 3,4-dihydroxybenzoic acid (D) and the proton of the ligand, respectively.

^b Ref. [35], $T = 25$ °C; $I = 0.2$ mol dm⁻³ KCl.

^c Ref. [35], $T = 30$ °C; $I = 0.1$ mol dm⁻³ KNO₃.

^d Ref. [35], $T = 25$ °C; $I = 1.0$ mol dm⁻³ NaClO₄.

^e The sigma (σ) value expresses the goodness of potentiometric data fitting using Hyperquad2008.

ization along the benzene ring. The system then tends to lose one proton of the para-hydroxyl group and rearrange the electron distribution [34]. According to these descriptions, the protonation equilibria of the ligands A and D are depicted in Fig. 1(a) and (b), respectively.

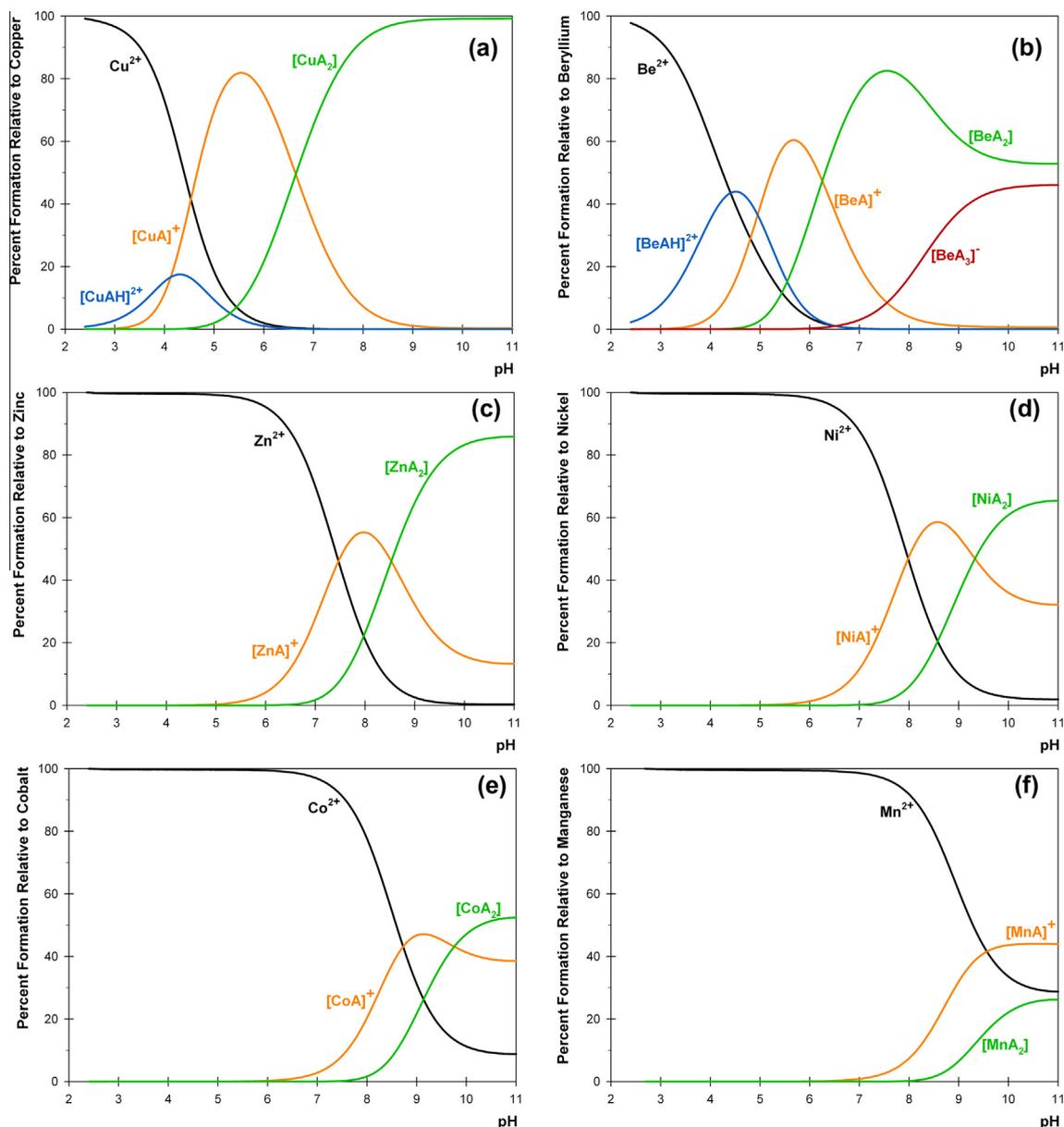


Fig. 6. Species distributions for binary system of 2-aminophenol (A) and the metal(II) ion: (a) Cu^{2+} ; (b) Be^{2+} ; (c) Zn^{2+} ; (d) Ni^{2+} ; (e) Co^{2+} ; and (f) Mn^{2+} ; at a 1:2.5 metal to ligand ratio, $T = 37 \pm 0.1$ °C and $I = 0.15$ mol dm^{-3} NaCl.

The obtained protonation constants were then introduced into the HySS2009 program to obtain the speciation diagrams of the deprotonated ligands (Fig. 2). These species distribution diagrams will be used to explain the occurrence of shifting on the UV–Vis spectra obtained for the qualitative investigation of ligand protonation. As presented in Fig. 3, ligand A has two optimum peaks (216 and 272 nm) at pH 2.5. As the pH of the solution was increased to 4.0, these peaks shifted to 213 and 278 nm, followed by the appearance of shoulder peaks at increasing wavelengths. Assessed by the species diagram in Fig. 2(a), these shifts are likely to be caused by the ionization of $[\text{AH}_2]^+$ species, which started to deprotonate, and 22.60% were converted into $[\text{AH}]$ species. With increasing pH, more $[\text{AH}]$ species were formed in the system (approximately 48.0% at pH 4.5) and a larger bathochromic shift to 226 and 282 nm can be observed in its spectra. Both peak heights kept increasing until pH 7.0, when 92.23% of ligand A appeared as $[\text{AH}]$ species. At pH 9.5, about 45% of this ligand lost its second proton to form $[\text{A}]^-$ species, thus its maximum peaks shifted to 214 and 291 nm.

UV–Vis spectra showing the shifts for the ligand D between pH 2.5 and 10.0 are presented in Fig. 4. At pH 2, three distinct optimum peaks at 221, 265 and 298 nm can be observed. These peaks are hypsochromically shifted to 218, 256 and 293 nm, respectively, as the pH is increased to about 5. Compared to the species that are formed at this pH (Fig. 2(b)), it is suggested that the alteration is caused by the presence of 79.24% of ligand D which has lost one proton and formed $[\text{DH}_2]^-$ species. When the pH is increased to 7.0, most species (97.42%) appear as $[\text{DH}_2]^-$, thus no change of the maximum wavelengths was found, instead an increase of the absorbance can be noticed. Another significant spectral change of ligand D can be observed at pH 9, where 70% of the ligand in the solution exists in its monobasic form $[\text{DH}]^{2-}$. At this pH, the spectra showed the disappearance of peak at 256 nm that previously occurred at pH 5.0. A new third peak at 301 nm also shows a red shift and has higher intensity. $[\text{DH}]^{2-}$ is predominant in the system at pH 10.0 and a new second peak occurred at 288 nm. In addition, its first peak has a broader shoulder, whereas its third peak has higher intensity.

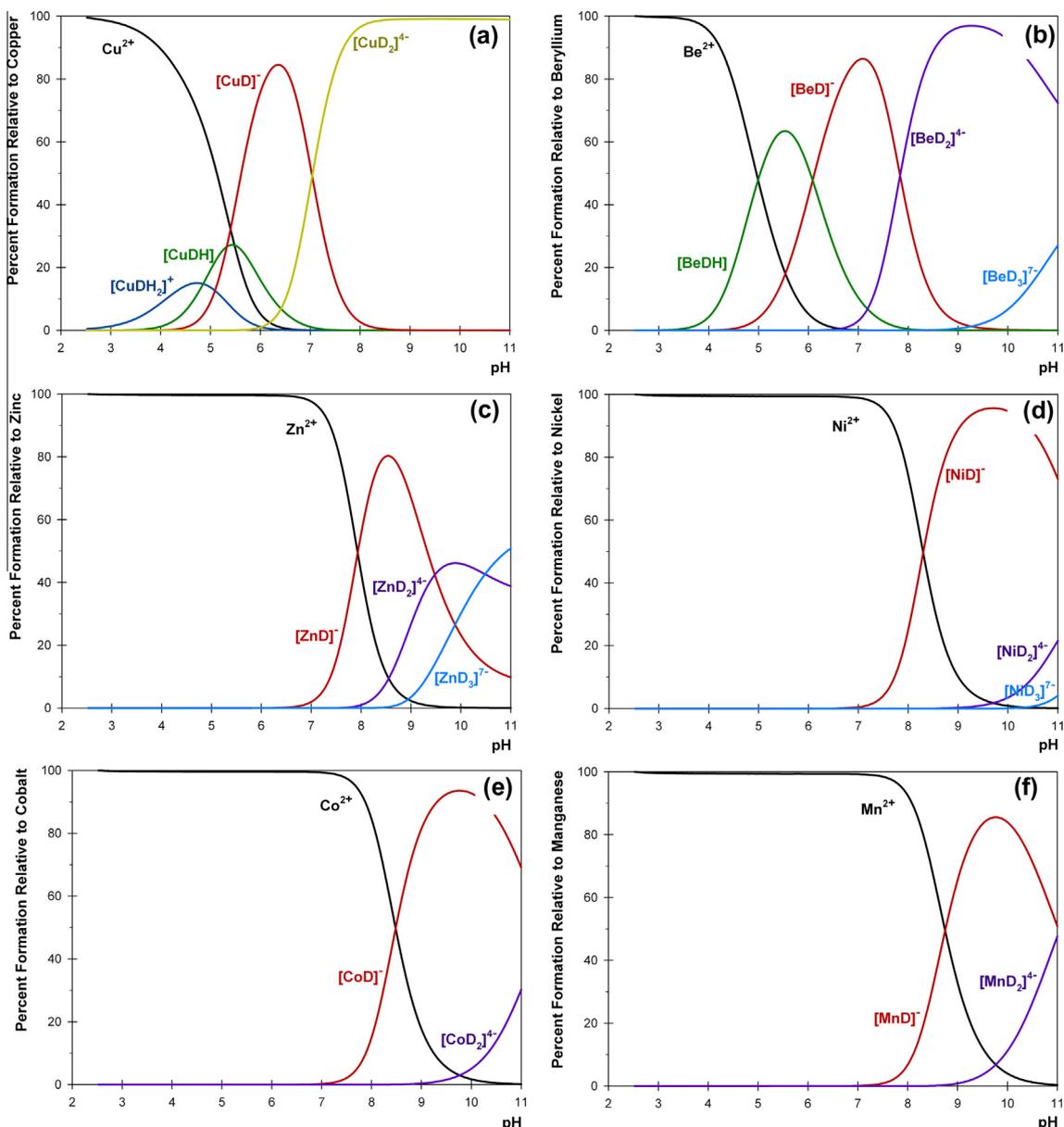


Fig. 7. Species distributions for the binary systems of 3,4-dihydroxybenzoic acid (D) and the metal(II) ions: (a) Cu^{2+} ; (b) Be^{2+} ; (c) Zn^{2+} ; (d) Ni^{2+} ; (e) Co^{2+} and (f) Mn^{2+} ; at a 1:2.5 metal to ligand ratio, $T = 37 \pm 0.1$ °C and $I = 0.15$ mol dm^{-3} NaCl.

3.2. Binary system of metal–ligand complexes

Several metal ions, where their atomic number increases following the trend: $\text{Be}^{2+} < \text{Mn}^{2+} < \text{Co}^{2+} < \text{Ni}^{2+} < \text{Cu}^{2+} < \text{Zn}^{2+}$, were used in this study. For the determination of the metal complex formation constant ($\log \beta$) of the binary system, solution c, that consists of metal ions and ligand A or D in various molar ratios (1:1, 1:2, 1:2.5 and 1:3) was titrated against carbonate free NaOH solution. Typical titration curves at a 1:2.5 metal to ligand ratio are shown in Fig. 5. For both ligands, it can be observed that there is a shifting of the buffer region to lower pH when metal ions are introduced into the system. The largest shifting occurred in the system containing Cu^{2+} , followed by systems containing Be^{2+} , Zn^{2+} , Ni^{2+} , Co^{2+} and Mn^{2+} . This shifting suggests that the formation of the metal–ligand complex begins as the ligand starts to deprotonate.

Several combinations of complex species that may have occurred during the complexation reaction were introduced into

the Hyperquad2008 program and the one that best fitted the experimental titration data was chosen. The results obtained from the refinement were expressed as overall formation constants ($\log \beta_{\text{MPAQH}_s}$ or $\log \beta_{\text{MPDRH}_s}$) and are summarized in Tables 3 and 4 for the systems with ligands A and D, respectively. In the binary system of the ligand A, Cu^{2+} appears to have the highest stability ($\log \beta_{\text{CuA}} = 8.48 \pm 0.01$). The stability constants for the metals in the binary system follow the order: $\text{Cu}^{2+} > \text{Be}^{2+} > \text{Zn}^{2+} > \text{Ni}^{2+} > \text{Co}^{2+} > \text{Mn}^{2+}$.

For the binary complexes of ligand A with metal ions, most cases showed that the maximum numbers of substituents attached to the metal ion ($[\text{MA}_2]$) is two. The presence of $[\text{MA}_3]^-$ occurred only in the system with Be^{2+} , which is due to the physical properties of this metal ion. Be^{2+} is known as a hard acid and has great coordination with hard bases, such as R-OH and R-NH₂ that are part of the ligand A [36]. In addition, Be^{2+} has the smallest ionic radius among all metal ions used in this study [37]. Its size allows other ligands that still have a lone electron to approach close

Table 5

Logarithms of the overall stability constants of ternary systems involving several metals ($\log \beta_{pqrs}$) at 37 ± 0.1 °C and $I = 0.15$ mol dm⁻³ NaCl.

Complex species	<i>p</i>	<i>q</i>	<i>r</i>	<i>s</i>	$\log \beta_{pqrs}^{a,b} \pm SD^c$	
					Hyperquad	Hypspec
<i>M = Cu²⁺</i>						
[MADH] ⁻	1	1	1	1	26.27 ± 0.05	28.24 ± 0.05
[MAD] ²⁻	1	1	1	0	19.58 ± 0.03	20.65 ± 0.07
($\sigma = 1.19$) ^d						
<i>M = Be²⁺</i>						
[MADH] ⁻	1	1	1	1	27.20 ± 0.02	27.23 ± 0.02
[MAD] ²⁻	1	1	1	0	19.12 ± 0.03	19.12 ± 0.04
($\sigma = 1.23$) ^d						
<i>M = Zn²⁺</i>						
[MADH] ⁻	1	1	1	1	22.44 ± 0.04	25.22 ± 0.04
[MAD] ²⁻	1	1	1	0	14.29 ± 0.02	14.96 ± 0.06
($\sigma = 1.23$) ^d						
<i>M = Ni²⁺</i>						
[MADH] ⁻	1	1	1	1	22.20 ± 0.02	22.96 ± 0.03
[MAD] ²⁻	1	1	1	0	13.17 ± 0.02	13.70 ± 0.03
($\sigma = 1.21$) ^d						
<i>M = Co²⁺</i>						
[MADH] ⁻	1	1	1	1	21.61 ± 0.01	20.99 ± 0.05
[MAD] ²⁻	1	1	1	0	13.11 ± 0.01	13.26 ± 0.03
($\sigma = 1.15$) ^d						
<i>M = Mn²⁺</i>						
[MADH] ⁻	1	1	1	1	21.00 ± 0.02	–
[MAD] ²⁻	1	1	1	0	11.56 ± 0.03	11.96 ± 0.03
($\sigma = 1.19$) ^d						

^a *p*, *q*, *r* and *s* represent the stoichiometric coefficients of the metal ion, 2-aminophenol (A), 3,4-dihydroxybenzoic acid (A) and proton ion, respectively.

^b No references were found for these complexes.

^c Standard deviation value of the last decimal place; ±95% confidence interval.

^d The sigma (σ) value expresses the goodness of potentiometric data fitting using Hyperquad2008.

enough to cause a strong ionic interaction. This interaction follows Coulomb's Law, where the force of attraction increases as the attracting species gets closer [38].

According to its speciation diagram (Fig. 6), the system with Cu²⁺ was able to form [CuA]⁺ species in more acidic pH (pH 3.5) compared to systems with other metal ions, where [BeA]⁺, [ZnA]⁺, [NiA]⁺, [CoA]⁺ and [MnA]⁺ species were found to form at pH 4.0, 5.5, 6.0, 7.0 and 7.5, respectively. Similarly, [CuA₂] species also started to form at pH 5.0 and achieved about 95% of [MA₂] at pH 8.0, while other metals started to form [MA₂] species at a higher pH (~5.0, 7.0, 7.5, 8.0 and 8.5 for [BeA₂], [ZnA₂], [NiA₂], [CoA₂] and [MnA₂], respectively).

A similar observation was also noticed in binary systems of the ligand D. As presented in Table 4, the largest stability value was obtained in the system containing Cu²⁺ ($\log \beta_{CuD} = 13.77$), followed by other metal–ligand systems ($\log \beta_{MD} = 13.52, 8.71, 8.06, 7.76$ and 7.36 for Be²⁺, Zn²⁺, Ni²⁺, Co²⁺ and Mn²⁺, respectively). Speciation diagrams of the binary systems containing ligand D (Fig. 7) confirm that these systems have at least two ligands attached to the metal ion. Ligand D begins to form [MD₂]⁴⁻ complexes at pH values of about 6.0, 7.0, 8.0, 9.0, 9.0 and 9.0 for systems containing Cu²⁺, Be²⁺, Zn²⁺, Ni²⁺, Co²⁺ and Mn²⁺, respectively.

In this work, the formation of complex species in a stepwise manner was also investigated. The log value of the stepwise association constants ($\log K_{MLn}$) were calculated from the following equations [27]:



$$\log K_{MLn} = \log \beta_{MLn} - (\log \beta_{MLn-1} + \log \beta_{MLn-2} + \dots) \quad (4)$$

where L refers to ligand A or D, and the subscript *n* denotes the number of ligands attached to the metal ion. It can be seen that the logK value of [MA₂]²⁻ species is smaller than that of [MA] species, for example $\log K_{CuA_2} (6.36) < \log K_{CuA} (8.48)$. A similar trend was also observed in the system containing ligand D ($\log K_{ML_3} < \log K_{ML_2} < \log K_{ML}$). Thus it can be concluded that the stepwise formation constant decreases with the increasing number of ligands attached to the metal ion. This phenomenon occurs possibly due to the reduction of the coordination number and the occurrence of steric effects at the metal ion after attachment of the first ligand.

Besides [ML], [ML₂] and [ML₃] species, it was also observed that at low pH, both ligand A and D may form a complex with Cu²⁺ and Be²⁺ in the protonated form, [MLH_n]. From the species distribution diagrams (Figs. 6 and 7), it is obvious that [CuAH]²⁺ and [BeAH]²⁺ species can be found between pH 2 and 6, while the species [CuDH₂]⁺, [CuDH] and [BeDH] can be found between pH 3.0 and 8.0. The logK value of the [MLH_n] species was found to be smaller than the logK value of the [ML] species ($\log K_{CuAH} = 3.03$; $\log K_{BeAH} = 3.50$; $\log K_{CuDH} = 6.34$; $\log K_{CuDH_2} = 2.57$; $\log K_{BeDH} = 6.85$). This confirms the basic theory that complexation of a bidentate ligand with a five-membered ring gives better stability, since the formation tendency achieves more by the enthalpy effect than by the entropy effect [39,40]. This greater enthalpy effect increases the crystal field strength, which means it increases the point of attachment of the ligand to the metal ion, imparting a higher chelating tendency to the latter [39,40]. Since the [MLH_n] species has a significantly weaker stability than the [ML] species, in the systems with less stable [ML] species, particularly the systems that contain Zn²⁺, Ni²⁺, Co²⁺ and Mn²⁺, the [MLH_n] species did not appear in the early pH range.

According to the observations, both ligands A and D form complexes with divalent metal ions in a similar manner. The overall result shows that the stabilities of the binary ligand–metal system decrease in the following order: Cu²⁺ > Be²⁺ > Zn²⁺ > Ni²⁺ > Co²⁺ > Mn²⁺. This trend in stability constants coincides with the order in the magnitude of the buffer region shifting, shown in Fig. 5. Moreover, this stability order agrees with the Irving–William series [41] and the results of Ahrlund et al. [42], where *a*-type metals, including the divalent metals used in this study, will form stable complexes with ligands that possess N, O and F atoms as the coordinating binding site. However, in terms of coordination ability, the binary systems with ligand A showed less stable coordination than the systems involving ligand D.

3.3. Ternary system of metal–ligand complexes

The obtained protonation constants of the ligands A and D, along with their complex stability constants with metal ions (Cu²⁺, Be²⁺, Zn²⁺, Ni²⁺, Co²⁺ and Mn²⁺) in the binary systems were introduced into the Hyperquad2008 program for determination of the stability constants of complexes in ternary systems. The refinement results are presented as the overall formation constants ($\log \beta_{MPqDrHs}$) in Table 5.

Again, the results suggest that the system with Cu²⁺ has the highest stability among all the ternary systems studied. The overall stability of the complex formed by the metal ion with two ligands ($\log \beta_{MAD}$) decreases in a similar order as that in the binary system: Cu²⁺ > Be²⁺ > Zn²⁺ > Ni²⁺ > Co²⁺ > Mn²⁺. The stability constant values were supported by the refinement from the spectrophotometric method using Hypspec, where the value differences are very small from the Hyperquad refinement results. These obtained stability constants of complex species in both binary and ternary systems then were used as the input in the HySS2009 program to predict the distribution of the involved species within the experimental pH range (Fig. 8). It turns out that most metal ions in the ternary systems started to form MADH at almost the same pH

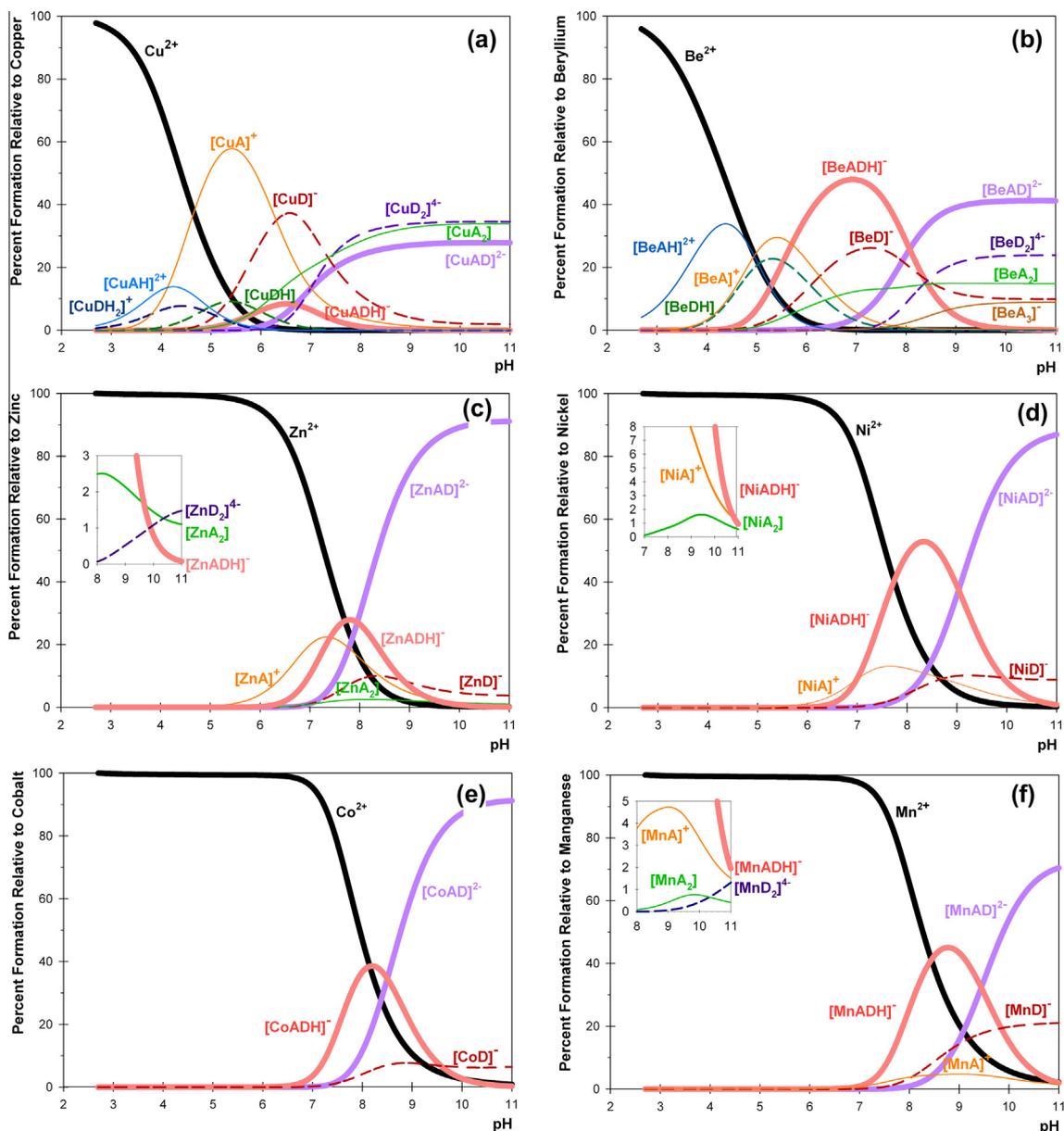


Fig. 8. Species distribution for ternary systems involving: (a) Cu^{2+} , (b) Be^{2+} , (c) Zn^{2+} , (d) Ni^{2+} , (e) Co^{2+} and (f) Mn^{2+} at a 1:1 metal to ligand ratio, $T = 37 \pm 0.1^\circ\text{C}$ and $I = 0.15 \text{ mol dm}^{-3} \text{ NaCl}$.

where the coordination of the binary ligand D began. The formation was quite small, but it played a role in initiating the formation of the ternary complex (MAD).

Fig. 8 also shows that ligand A coordinated to metal ions and formed [MAH] or [MA] species at a lower pH than ligand D, which suggests that ligand A acted as the primary chelating compound in the systems. This is because amino group ($-\text{NH}_2$) in ligand A makes it a hard Lewis base which leads to early deprotonation. At higher pH, some metal–ligand A complexes appeared to lose their coordination, leading to the formation of metal–ligand D. This ligand substitution process was expected since in the binary system studies ligand D showed larger stability constant values which suggests stronger metal–ligand coordination than for ligand A. Therefore, the interaction of the metal with the primary ligand was initiated at the beginning, then coordination of these donor groups was replaced by the secondary ligand (ligand D).

Spectral measurements for all ternary systems involving one divalent metal ions (Cu^{2+} , Be^{2+} , Zn^{2+} , Ni^{2+} , Co^{2+} or Mn^{2+}) and two

ligands (A and D) have been performed in the UV–Vis range wavelength and the results are given in Table 6. The spectral bands were observed at an initial pH of 2.5 and pH 11.0, where at pH 11.0 the formation of MAD species was observed at the highest concentration, based on the speciation diagram. The observed change in the spectral bands also confirmed the formation of the MAD complex in the system.

At an initial pH of 2.5 some peaks were observed: (1) the aromatic ring of the ligand gives a spectrum which was observed at 210–244 nm; (2) the observed band at 260–267 nm for all systems refers to the $\pi-\pi^*$ electronic transition of the O donor atom from the ligand D to the metal ion, while the N donor atom from ligand A give rises to a band at 298–312 nm – both ligands used are weak field ligands, in which the ligand donates an electron to bind with the metal ion; (3) the $d-d$ transition band of the metal ion was clearly observed at the initial pH since the free metal ion was still of a high concentration. For Cu^{2+} (d^9) the bands observed at 414 and 620 nm are due to the electronic transitions ${}^2\text{A}_{1g} \rightarrow {}^2\text{B}_{1g}$ and

Table 6
UV–Vis data of ternary systems involving several metals.

Metal ions	λ_{\max} (nm)		Assignment
	pH 2.5	pH 11.0	
Cu ²⁺	210–239	211–241	aromatic ring
	266	270	π – π^*
	301	316	π – π^*
	414	440	$^2A_{1g} \rightarrow ^2B_{1g}$
	620	–	$^2E_g \rightarrow ^2B_{1g}$
Be ²⁺	210–238	211–238	aromatic ring
	266	275	π – π^*
	300	308	π – π^*
	436	444	red shifting
Zn ²⁺	212–239	212–240	aromatic ring
	260	264	π – π^*
	312	319	π – π^*
	439	442	red shifting
Ni ²⁺	210–237	210–241	aromatic ring
	267	274	π – π^*
	298	300	π – π^*
	446	451	$^3A_{2g}(F) \rightarrow ^3T_{1g}(P)$
	521	–	$^3A_{2g}(F) \rightarrow ^3T_{1g}(F)$
Co ²⁺	211–244	211–250	aromatic ring
	267	270	π – π^*
	299	311	π – π^*
	445	436	$^4A_{2g}(F) \rightarrow ^4T_{1g}(P)$
	654	–	$^4A_{2g}(F) \rightarrow ^4T_{1g}(F)$
Mn ²⁺	210–239	211–250	aromatic ring
	265	268	π – π^*
	310	307	π – π^*
	447	430	$^6A_{1g}(S) \rightarrow ^4T_{1g}(G)$

Table 7
The $\Delta \log K_M$ and $\log X$ values of ternary systems involving 2-aminophenol (A) and 3,4-dihydroxybenzoic acid (D).

Metal ions	$\Delta \log K_M$	$\log X$
Cu ²⁺	–2.67	–0.19
Be ²⁺	–2.52	0.68
Zn ²⁺	0.33	3.31
Ni ²⁺	0.37	5.12
Co ²⁺	1.27	6.06
Mn ²⁺	0.67	3.96

$^2E_g \rightarrow ^2B_{1g}$, respectively. Be²⁺ (*s*²) and Zn²⁺ (*d*¹⁰) were found not to have any orbital transition. The observed bands at 446 and 521 nm for Ni²⁺ (*d*⁸) are attributed to the transitions $^3A_{2g}(F) \rightarrow ^3T_{1g}(P)$ and $^3A_{2g}(F) \rightarrow ^3T_{1g}(F)$, respectively. For Co²⁺ (*d*⁷), the orbital transition $^4A_{2g}(F) \rightarrow ^4T_{1g}(P)$ was observed at 445 nm, while the transition $^4A_{2g}(F) \rightarrow ^4T_{1g}(F)$ was observed at 654 nm. For Mn²⁺ (*d*⁵), the transition $^6A_{1g}(S) \rightarrow ^4T_{1g}(G)$ was observed at 447 nm.

At pH 11.0, the MAD species in all systems were found in high concentration and caused a shifting of the bands. The changes of the spectra were observed as follows: (1) the aromatic ring band shifts to higher wavelength (210–250 nm); (2) the band corresponding to the π – π^* electronic transition of the O donor atom of the ligand shifts to 264–275 nm, while the band corresponding to the N donor atom shifts to 300–316 nm; (3) shifts for the electronic transitions of the metal ions. For Cu²⁺ (*d*⁹), the band for the $^2A_{1g} \rightarrow ^2B_{1g}$ transition shifts to 440 nm, while the band of $^2E_g \rightarrow ^2B_{1g}$ disappears. For Ni²⁺ (*d*⁸), the band attributed to the transition $^3A_{2g}(F) \rightarrow ^3T_{1g}(P)$ shifts to 451 nm and the other band also disappears. For Co²⁺ (*d*⁷), the orbital transition $^4A_{2g}(F) \rightarrow ^4T_{1g}(P)$ shifts to a lower wavelength (436 nm). For Mn²⁺ (*d*⁵), the transition $^6A_{1g}(S) \rightarrow ^4T_{1g}(G)$ also shifts to a lower wavelength (447 nm). Some of the *d*–*d* transition bands disappear, possibly due to their low

intensity, the bands being overlapped by the ligand donor band which has a higher intensity.

Further analysis of the complexation equilibria in the ternary systems was done by calculating $\Delta \log K_M$ (Eq. (5)), which is the relative stability of the ternary complex (MAD) compared to the complex stability in the corresponding binary system (MA and MD) [43].

$$\Delta \log K_M = \log K_{MAD}^{MA} - \log \beta_{MD} = \log K_{MAD}^{MD} - \log K_{MA} \quad (5)$$

A positive value of $\Delta \log K_M$ indicates the tendency of the metal to form a ternary complex rather than a binary complex [44]. The calculated $\Delta \log K_M$ values of ternary systems involving ligand A, ligand D and several divalent metal ions (Cu²⁺, Be²⁺, Zn²⁺, Ni²⁺, Co²⁺ and Mn²⁺) are given in Table 7. From this table, it can be seen that the $\Delta \log K_M$ values for all the systems are negative. These values indicate that formation of a binary metal complex (MA or MD) is preferred over that of the ternary complex (MAD). One possible reason is the occurrence of steric hindrance, where attachment of the primary ligand to the metal ion increases the bulkiness of the complex structure. Thus it prevents the secondary ligand from coordinating and forming a ternary complex. Besides, the secondary ligand has less of a chance to interact with the chelated-metal ion due to reduction of the coordination sites at the metal ion and electrostatic effect between the metal and secondary ligand.

It is also observed that some divalent metal ions which show a higher stepwise stability with ligand D ($\log \beta_{MD}$) tend to have a more negative $\Delta \log K_M$. The order of stability in the binary system (both $\log \beta_{MD}$ or $\log \beta_{MA}$) was found to be almost opposite to the order of $\Delta \log K_M$ ($Co^{2+} > Mn^{2+} > Ni^{2+} > Zn^{2+} > Be^{2+} > Cu^{2+}$). It suggests that the formation of stable ternary complexes is more difficult in systems with a very stable binary complex. However, once a ternary complex is formed, it has greater stability than those of the binary systems.

The stability of ternary complexes can also be quantitatively analyzed by their $\log X$ values, which were calculated using Eq. (6). This parameter was used to predict the tendency of one mole of the binary complex MA₂ and one mole of the binary complex MD₂ to form two moles of the ternary complex MAD [45,46].



The results for $\log X$ are given in Table 7. Positive values of $\log X$ were found for most metal ions, which suggests that the formation of ternary complexes containing two different ligands (MAD) is more favorable than complexes with two similar ligands (MA₂ or MD₂). Formation of a ternary complex containing two similar ligands (MA₂ or MD₂) has greater strain of coordination, thus the system prefers to form ternary complexes with different ligands (MAD). In contrast, the Cu²⁺ ion was the only metal ion that gave a negative value, which means it also has a strong tendency to form MA₂ or MD₂ complexes. As shown in Fig. 8, CuA₂ and CuD₂ were stable and formed together along with CuAD.

4. Conclusions

Two chelating agents, 2-aminophenol (ligand A) and 3,4-dihydroxybenzoic acid (ligand D), were employed in this work to study the complex formation of these ligands with divalent metal ions (Cu²⁺, Be²⁺, Zn²⁺, Ni²⁺, Co²⁺ and Mn²⁺) at 37 °C and with an ionic strength of 0.15 mol dm^{–3} NaCl. Ligand A possesses an amino group (–NH₂) and a hydroxyl group (–OH) that were protonated at log *K* 4.53 and 9.58, respectively. In contrast, ligand D has three protonation sites, occurring at log *K* 4.42, 8.62, and 12.75, which correspond to the carboxylic, para-hydroxyl and meta-hydroxyl groups, respectively.

Divalent metal ions, forming binary complexes with ligands A or/and D in both binary and ternary systems, have stability in the following order: $\text{Cu}^{2+} > \text{Be}^{2+} > \text{Zn}^{2+} > \text{Ni}^{2+} > \text{Co}^{2+} > \text{Mn}^{2+}$. This order is in agreement with the buffer region shifting that occurred in potentiometric titration curves, where the furthest and closest shifting can be seen in systems containing Cu^{2+} and Mn^{2+} , respectively. Based on its overall formation constant values, ligand D showed a better chelating ability with metal ions than ligand A. However, based on speciation diagrams of the complexes in the ternary systems, it is suggested that ligand A acted as the primary ligand in these systems and was coordinated to metal ions at lower pH.

Investigations of the stability of the ternary complexes relative to their corresponding binary complexes were also carried out in this work by calculating $\Delta \log K_M$ and $\log X$ values. Negative values of $\Delta \log K_M$ were obtained in all the systems studied, which indicated that formation of binary metal complexes (MA or MD) is more preferable than formation of ternary complexes (MAD). However, larger values of $\log \beta_{MAD}$ compared to $\log \beta_{MA}$ and $\log \beta_{MD}$ suggests that the metal–ligand complexes that are formed in the ternary systems have greater stability than those of the binary systems. On the other hand, calculation of $\log X$ gave positive values for most metal ions studied, which indicates that the formation of ternary complexes containing two different ligands (MAD) is more preferable than complexes involving two similar ligands (MA_2 or MD_2).

Acknowledgements

Financial support by the National Science Council of Taiwan (NSC 102-2221-E-011-079) and the National Taiwan University of Science and Technology (101H451403) is greatly appreciated.

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