Ternary Complexes of Cobalt(II) involving Nitrilotriacetic Acid and Some Biologically Active Ligands

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Abstract

Ternary complexes of cobalt(II) with nitrilotriacetic acis as a primary ligand and some selected mono- and dicarboxylic acids as secondary ligands have been investigated by the potentiometric technique at T = 30ºC and I = 0.1 mol dm⁻³. The ternary complexes are formed in a stepwise mechanism. Confirmation of the ternary complexes in solution has been carried out using conductometric measurements. The solid complexes of cobalt(II) involving nitrilotriacetic acid as a primary ligand and phthalic acid or benzoic acid as a secondary ligands were prepared and characterized by elemental analysis, IR and thermogravimetric analysis.

Keywords: Ternary complexes, nitrilotriacetic acid, potentiometric technique, thermogravimetric analysis.

Introduction

Many authors investigated coordination compounds due to their chemical, biological, environmental and catalytic importance[1–2]. Ternary complexes of metal (II) containing nitrogen and oxygen-donor ligands have received much attention recently as they can display exceptionally high stability[3–5]. Ternary metal complexes have been recently studied due to their ability as metal systems for metal–protein complexes such as metallo-enzymes. They received particular attention and have been employed in mapping protein surfaces[6], as probes for biological redox centers[7] and in protein capture for both purification[8] and study[9,10]. Study of the structure of model ternary complexes provides information about how biological systems achieve their specificity and stability, as well as strategies to improve these features for biotechnological applications.

Ternary metal complexes of nitrilotriacetic acid (NTA) have been widely adopted in biology and are gaining increasing uses in biotechnology, particularly in protein purification techniques known as immobilized metal ion affinity chromatography (IMAC)[11]. Nitrilotriacetic acid has gained, recently, popularity as metal chelator in IMAC. Because of the great biological applications, much of the studies on the coordination of (H_3NTA) with transition metal to form binary or ternary complexes have been done in neutral or slightly basic media, where such ligands are the deprotonated acids (NTA^{3-})[12–16]. On the other hand, synthesis of different types of complexes using various amino acids is becoming increasingly important primarily because of the various applications of such complexes in biological fields[17–19].

The formation constants of the ternary complexes of Ni(II), Cu(II) and Zn(II) with nitrilotriacetic acid as a primary ligand and glycine as secondary ligand were determined by Hopgood and Augelici[14]. Stabilities of ternary complexes of cobalt(II), nickel(II), copper(II) and zinc(II) involving aminopolycarboxylic acids and heteroaromatic *N*-bases as primary ligands and benzohydroxamic acid as a secondary ligand have been determined at 25°C and an ionic strength of 0.1 mol dm⁻³ KNO_3 by the Irving-Rossotti technique[20].

In the present work, we are reporting the preparation of ternary complexes of cobalt(II) with nitrilotriacetic acid as primay ligand and some mono-and dicarboxylic acids as secondary ligands which may be of biochemical and analytical importance. The characterization of these complexes has also been carried out in order to establish the stability of these complexes and coordinating power of ligands.

Material and Methods

Experimental Section: Materials and Solutions : Cobalt(II) nitrate, nitrilotriacetic acid (NTA) were from Fluka. Mono-and dicarboxylic acids were analytical-grade (Aldrich or Merck) products. Stock solutions of cobalt(II) nitrate were prepared in deionized water. A carbonate-free sodium hydroxide (titrant, prepared in 0.1 mol dm⁻³ $NaNO_3$ solution) was standardized potentiometrically with KH phthalate (Merck AG). A nitric acid solution (0.04 mol dm⁻³) was prepared and used after standardization. Sodium hydroxide, nitric acid and sodium nitrate were from Aldrich.

Apparatus and Procedure: The titrations were performed at (30 $\pm$ 0.1) °C in a double-walled cell fitted with a thermostat. A Schott CG 825 pH meter using a glass electrode was used to monitor the pH changes. The titrant (CO_2-free standard NaOH) was added to the titration cell, and the pH changes were monitored through the pH meter. The pH meter was calibrated with standard buffer solutions (pH 4.0 and 10.0) before the pH

measurements. The ionic strength was kept constant (0.10 mol dm^{-3}) using a NaNO$_3$ solution, and a total volume of 50 cm^3 was used for each titration.

For the study of ternary (1:1:1) complexes, the different solutions titrated were as follows: 0.004 mol dm^{-3} HNO$_3$ + 0.1 mol dm^{-3} NaNO$_3$ (a), solution a + 0.001 mol dm^{-3} NTA (b), solution b + 0.001 mol dm^{-3} Co(II) ion (c), solution a + 0.001 mol dm^{-3} mono-and dicarboxylic acids (d), solution d + 0.001 mol dm^{-3} Co (II) ion (e), and solution a + 0.001 mol dm^{-3} Co(II) ion + 0.001 mol·dm^{-3} NTA + 0.001 mol dm^{-3} mono-and dicarboxylic acids (f). The ternary metal complex solutions under investigation do not show any precipitation up to the pH value corresponding to complete complex formation, which reveals that these ternary complexes have no tendency to form hydroxo complex species. In analyzing the titration data for the determination of the proton dissociation constants of the free ligands and the stability constants of binary and ternary metal-ligand complexes in solution, Bjerrum-Calvin's pH titration technique[21, 22] as adopted by Irving and Rossotti [23, 24] for binary systems and by Chidambaram and Bhattacharya [25] for ternary systems, has been used at (30 $\pm$ 0.1) ºC.

Conductometric titrations were followed with a HANNA HI 9835, Microprocessor conductivity/TDS Meter. The following mixture was titrated conductometrically against a 0.10 mol dm^{-3} NaOH solution: 0.01 mol dm^{-3} Co (II) (10 cm^3) + 0.01 mol dm^{-3} NTA (10 cm^3) + 0.01 mol dm^{-3} mono-and dicarboxylic acids (10 cm^3).

All two mixed-ligand complexes containing NTA were derived from diaquanitrilotriacetatocobalt(II), Na[Co^{II}(NTA)(H$_2$O)$_2$][26].

A 2.92 gm portion (0.01 mol) of complex, Na[Co^{II}(NTA)(H$_2$O)$_2$], as a starting material was suspended in 25 ml of water and slightly warmed in the water bath with stirring, then 1.22 gm (0.01 mol) of benzoic acid or 1.66 gm (0.01 mol) of phthalic acid were added. When the complexes were then gradually dissolved in water, the color of the solution turned from pinkish violet to pink. About 50 ml of ethanol were then added, when the solution was cooled, pink crystals out, filtered and dried at room temperature.

Instrumentation: All measurements were carried out at the micro-analytical laboratories of Cairo University, and the National Center for Research Tripoli-Libya. C, H and N were determined by Vario El Elementar. IR spectra of the solid complexes were recorded on a Jasco FTIR-300 E Fourier Transform Infrared Spectrometer, using KBr discs in the range 400–4000 cm^{-1} and CsI technique in the range 200–500 cm^{-1}. The Thermogravimetric analysis of the complexes under investigation were preformed using NETZSCH TG 209. Thermo- gravimetry (TG) is the method in which the mass of the sample is monitored as a function of temperature. Generally, mass changes are caused by loss of volatile components (such as water) from the sample by heating.

Results and Discussion

Ternary complexes formation of cobalt(II) involving nitrilotriacetic acid as primary ligand and some mono-and dicarboxylic acids as secondary ligands in aqueous medium: Representative pH-metric equilibrium titration curves for the free and Co(II) complexed ligands are depicted in figures-1, 2 and 3. When a solution contains two different ligands and a metal ion, they may exist in equilibria in which either i. both the ligands may combine with the metal ion simultaneously or ii. the two ligands may be combined one by one at different pH. As is evident from the titration curves in the present study, the addition of two ligands is stepwise. It was deduced that NTA interacts first with the Co(II) ion, followed by the interaction with the mono- and dicarboxylic acids; that is, the ternary complex formation could be considered in stepwise complexation equilibria, i.e., the formation of a ternary complex can be represented by the stepwise equilibria;
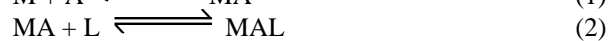
$$M + A \rightleftharpoons MA \quad\quad\quad (1)$$
$$MA + L \rightleftharpoons MAL \quad\quad\quad (2)$$
$$K_{MAL}^{MA} = [MAL] / [MA] [L] \quad\quad\quad (3)$$

where M = Co(II), A represents the primary ligand (NTA) and L represents the secondary ligand (mono-and dicarboxylic acid); for instance, examining figure-1, one may observe that the curves obtained for the different 1:1:1 ternary complex solutions (curve f) overlap with the titration curve of the 1:1 binary Co(NTA)- (curve c) at low pH values and a divergence of the ternary complex titration curve from that of the binary Co(NTA)- is observed at higher pH. This shows the coordination of the phthalic acid to the Co(NTA)-binary complex in a stepwise manner as represented by the following equations:

$$Co(II) + (NTA) \rightleftharpoons Co(NTA) \quad\quad (4)$$
$$Co(NTA) + phthalic\ acid \rightleftharpoons Co(NTA)$$
$$(phthalic\ acid) \quad\quad (5)$$
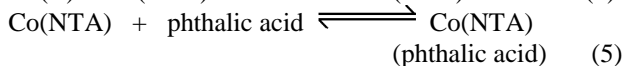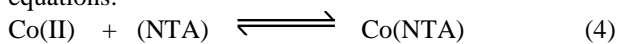
Part of the information required for determining the stability constants of ternary complexes is the protonation constants of the ligands under study. Therefore, prior to determining the stability constants of mixed complexes, the protonation constants of the ligands under study are determined. From the titration curves of the solutions a, b and d, the average number of protons associated with the ligands, $\overline{n}_H$, was calculated using equation 6, where y is the number of dissociable protons. V_a, V_b, and V_d are the volumes of NaOH consumed to reach the same pH values in curves a, b, and d, respectively. E^o and N^o are the concentrations of HNO$_3$ and NaOH, respectively. T_L^o is the initial total molar concentration of the primary ligands and secondary ligands studied in the titrated solution = 1.0 x 10^{-3} mol dm^{-3}, and V^o is the original volume (50 cm^3).

$$\overline{n}_H = y - \frac{(V_b\, or\, V_d - V_a)(E^o + N^o)}{(V_o + V_a)T_L^o} \quad\quad (6)$$

The calculated protonation constants are reported in table-1. However, the obtained protonation constants are in fairly good agreement with literature values obtained under similar experimental conditions[21-23]. The first ionizations, pK_{a1}, for NTA and the secondary ligands investigated are very low ($\leq$ 2.30), and dissociate in strongly acidic solutions. Therefore, these values could not be measured and were not used in the calculations.

Table-1
Acidity Constant of Ligands in Aqueous Solution at 30ºC and I = 0.1 mol dm^{-3} (NaNO$_3$)

Ligands	pK_{a1}	pK_{a2}	pK_{a3}
NTA		2.48 ±0.04	9.71±0.07
Phthalic acid	2.80 ± 0.03	5.43 ± 0.06	00
Benzoic acid	4.26 ± 0.06	00	00
Salicylic acid	2.68 ± 0.06	13.40*	00
Succinic acid	4.14 ± 0.03	5.73 ± 0.06	00
Malonic acid	2.86 ± 0.04	5.68 ± 0.05	00
Malic acid	3.34 ± 0.03	5.23 ± 0.06	00
Oxalic acid	00	4.32 ± 0.04	00
Tartaric acid	3.08 ± 0.02	4.39 ± 0.05	00
Glycine	00	9.65 ± 0.07	00
Alanine	00	9.81 ± 0.06	00
Valine	00	9.69 ± 0.05	00
Tryptophan	00	9.47 ± 0.08	00
Aspartic acid	00	9.83 ± 0.05	3.94 ± 0.04
Glutamic acid	00	9.74 ± 0.06	4.19 ± 0.06
Histidine	00	9.18 ± 0.08	6.30 ± 0.05

On the other hand, the horizontal distance between curves c and f (Figure-1) was determined and used for calculation of the average number of moles of the secondary ligand, $\bar{n}_{mix}$, from the following equation:

$$\bar{n}_{mix} == \frac{(V_f - V_c)[(E^o + N^o) + T_L^o(y - \bar{n}_H)]}{(V_o + V_c)[T_{M(primaryligand)}^o]\bar{n}_H} \quad (7)$$

where V_f and V_c are the volumes of NaOH consumed to reach the same pH value in the curves f and c, respectively. $[T_{M(primaryligand)}^o]$ is the concentration of the binary complex which is equivalent to the initial Co(II) ion concentration (T_M^o); $\bar{n}_H$ is the average number of protons associated with the secondary ligand; T_L^o is the initial concentration of the secondary ligand; and y is the number of dissociable protons per molecule of the secondary ligand. V_o, E^o, and N^o have the same meaning as mentioned before.

Since $E^o + N^o \gg T_L^o$

$$\bar{n}_{mix} == \frac{(V_f - V_c)[E^o + N^o]}{(V_o + V_c)[T_{M(primaryligand)}^o]\bar{n}_H} \quad (8)$$

From the values of $\bar{n}_{mix}$ so obtained, the free secondary ligand exponent, pL'_{mix} was calculated using the equation

$$pL'_{mix} = \log\left\{\frac{1 + 10^{pK_{a2}}(1/10^{pH})}{T_L^o - \bar{n}_{mix}T_M^o} \cdot \frac{(V_o + V_f)}{V_o}\right\} \quad (9)$$

Here, pK_{a2} is the second dissociation constants of the secondary ligands. All other terms have the same meaning as defined above. The formation curves corresponding to the different ternary complexes under investigation were constructed by plotting $\bar{n}_{mix}$ versus pL'_{mix}. At $\bar{n}_{mix}$ equals 0.5, the pL'_{mix} value is the corresponding stability constant of the ternary complex formed in the solution (1:1:1). The stability constants for ternary complexes have been calculated at 30ºC and I = 0.1 mol dm^{-3} and are tabulated in table-2.

Table-2
Formation Constant of the Ternary Complexes of Co(II) Involving NTA as a Primary Ligands and Amino Acid or Aliphatic and Aromatic acid as a Secondary Ligand at 30 ºC and I = 0.1 mol dm^{-3} (NaNO$_3$)

Ligands	$\log K_{Co(NTA)(L)}^{Co(NTA)}$
Phthalic acid	6.83 ± 0.07
Benzoic acid	6.41 ±0.04
Salicylic acid	6.25 ± 0.07
Succinic acid	6.67 ± 0.07
Malonic acid	6.35 ± 0.05
Malic acid	5.32 ± 0.03
Oxalic acid	5.85 ± 0.08
Tartaric acid	5.37 ± 0.04
Aspartic acid	6.64 ± 0.03
Glutamic acid	6.29 ± 0.06
Histidine	6.48 ± 0.07
Phenylalanine	5.53 ± 0.07
Alanine	5.37 ± 0.03
Valine	5.82 ± 0.07
Glycine	5.42 ± 0.06

In figure-4, a conductometric titration curve for the ternary complex of Co(II) with NTA and phthalic acid is displayed, as representative. The titration curve shows an initial decrease and an inflection at a = 2 (a = moles of base added per mole of ligand). This probably corresponds to the neutralization of H$^+$ ions originating from the formation of the Co(II) + NTA binary complex. In the 3 $\geq$ a $\geq$ 2 range, the conductance increases slightly due to the formation of a ternary complex associated with the release of a H$^+$ ion from phthalic acid. Beyond a = 3, the conductance increases appreciably due to the presence of an excess of NaOH.

Preparation of solid complexes of the ternary complexes of cobalt(II) involving ntrilotriacetic acid and phthalic acid or benzoic acid: The elemental analysis of the prepared complexes is illustrated in table -3. The obtained data are in

good agreement with the required for the suggested structure of all prepared complexes.

The infrared absorption spectra were measured to confirm whether the carboxylate groups were coordinate or not (table - 4). i. The spectra of the solid complexes exhibited bands in the 3520 - 3253 cm^{-1} regions, which may be attributed to νOH of the coordinated water molecules[27]. Ii. In the spectra of all studied complexes the νOH $_{acid}$ band in the free ligand completely disappeared and a new carboxylate band ($ν_{COO}$) appeared in the region 1464-1431 indicating that the carboxylic group of NTA participate in the coordination with the metal ions through deprotonation. all the spectra of the complexes studies showed asym-($ν_{COO}$-Co) band in the range 1562 to 1659 cm^{-1} as shown in table-4. Iii. The assignment of the M-N and M-O stretching frequencies (table -4) is supported by the work of Condrate and Nakamoto[28] who located the M-N stretching frequencies for some divalent metal glycine complexes in the range 628 - 445 cm-1 and M-O stretching bands between 420 and 280 cm^{-1}.in the present spectra of all the solid complexes

studies show the moderate band at 545 cm^{-1}, which is due to Co-O bond in the complexes.

Thermogravimetric analysis of the two complexes were carried out in order to confirm the structure and that the water present is coordinated to the central metal atom. The thermogram of the complex, $Na_3[Co^{II}(NTA)(P)(H_2O)]$, shows that weight loss (11.97) begins 189.1^{o}C corresponding to the loss of one coordinated water molecules and one carboxylate group (calcd. 12.40). Also, The thermogram of the complex, $Na_2[Co^{II}(NTA)(B)(H_2O)_2]$, shows that weight loss (8.23) begins 185.2 ^{o}C corresponding to the loss of two coordinated water molecules (calcd. 8.0). From the above data, the proposed structures of the ternary complexes of cobalt(II) involving nitrilotriacetic acid as a primary ligand and phthalic acid or benzoic acid as a secondary ligands are $Na_3[Co^{II}(NTA)(P)(H_2O)]$, and $Na_2[Co^{II}(NTA)(B)(H_2O)_2]$, respectively as follow:

Table-3
Analytical data for the Co^{II}-complexes

Compound	Color	Found /Calculated		
		C %	H %	N %
$Na_3[Co^{II}(NTA)(P)(H_2O)]$	Pink	33.34	2.49	2.72
		33.73	2.41	2.81
$Na_2[Co^{II}(NTA)(B)(H_2O)_2]$	Pink	34.09	3.21	3.27
		34.67	3.33	3.11

Table -4
Assignment of bands in the IR – Spectra of the metal complex

$Na_3[Co^{II}(NTA)(P)(H_2O)]$	$Na_2[Co^{II}(NTA)(B)(H_2O)_2]$	Assignment
3516 m	3520 m	ν OH$^-$ water
2970 s	2967 s	ν NH
1658 w	1659 w	ν C = O
1627 m	1627 w	ν C = O
1464 s	1464 s	ν asy COO$^-$
1433 s	1431 s	ν sym COO$^-$
1124 s	1123 s	ν C – N
552 w	545 w	ν C – H

s = sharp, m = medium, w = weak.
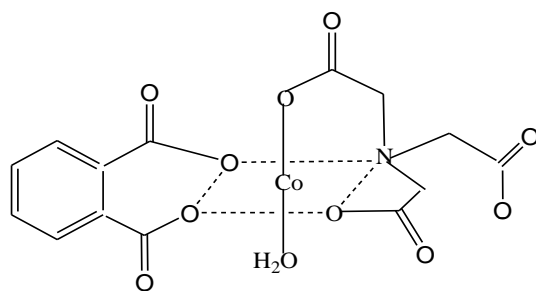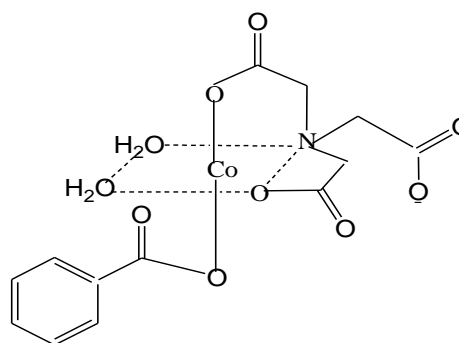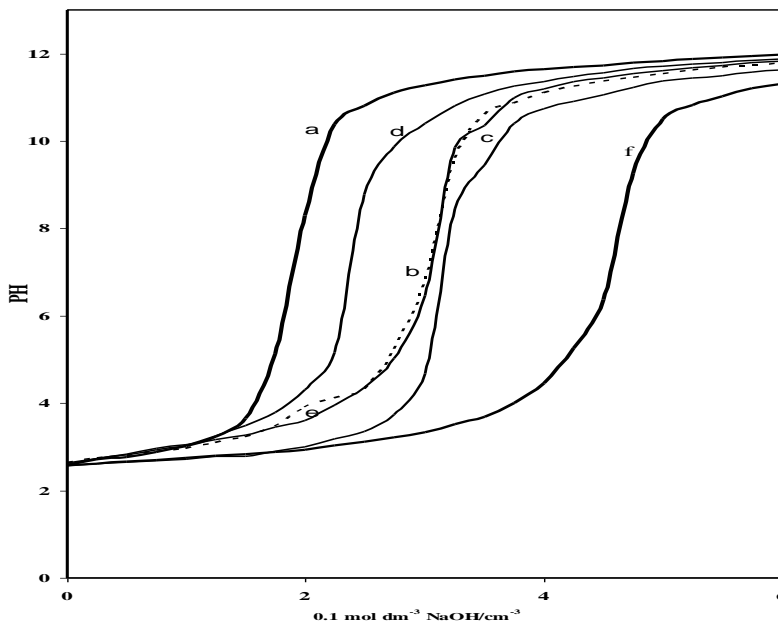

$Na_3[Co^{II}(NTA)(P)(H_2O)]$ $Na_2[Co^{II}(NTA)(B)(H_2O)_2]$

Figure-1
Potentiometric titration curves for the Co(II) + NTA + phthalic acid system at 30 ºC and *I* = 0.1 mol·dm^{-3} NaNO$_3$: 0.004 mol dm^{-3} HNO$_3$ + 0.1 mol dm^{-3} NaNO$_3$ (a), solution a + 0.001 mol dm^{-3} NTA (b), solution b + 0.001 mol dm^{-3} Co(II) ion (c), solution a + 0.001 mol dm^{-3} phthalic acid (d), solution d + 0.001 mol dm^{-3} Co(II) ion (e), and solution a + 0.001 mol dm^{-3} Co(II) ion + 0.001 mol dm^{-3} NTA + 0.001 mol dm^{-3} phthalic acid (f)
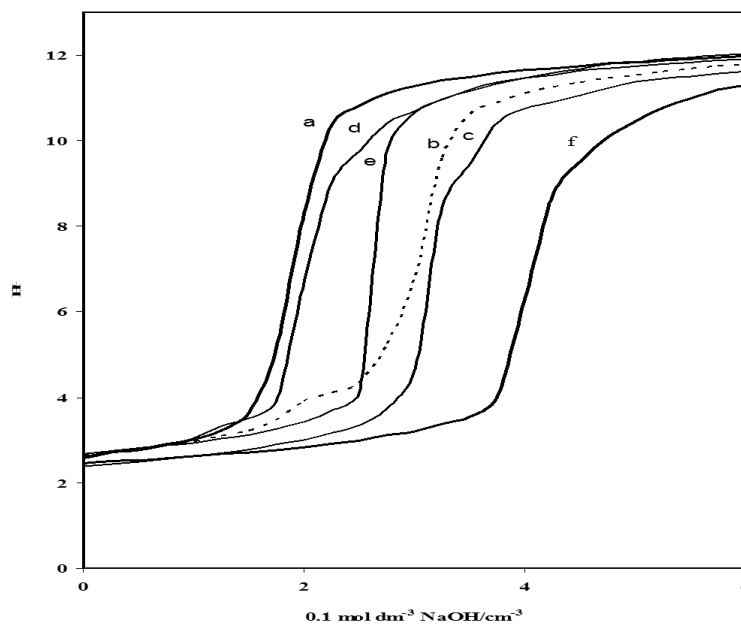

Figure-2
Potentiometric titration curves for the Co(II) + NTA + benzoic acid system at 30 ºC and *I* = 0.1 mol dm^{-3} NaNO$_3$: 0.004 mol dm^{-3} HNO$_3$ + 0.1 mol dm^{-3} NaNO$_3$ (a), solution a + 0.001 mol dm^{-3} NTA (b), solution b + 0.001 mol dm^{-3} Co(II) ion (c), solution a + 0.001 mol dm^{-3} benzoic acid (d), solution d + 0.001 mol dm^{-3} Co(II) ion (e), and solution a + 0.001 mol dm^{-3} Co(II) ion + 0.001 mol dm^{-3} NTA + 0.001 mol dm^{-3} benzoic acid (f)
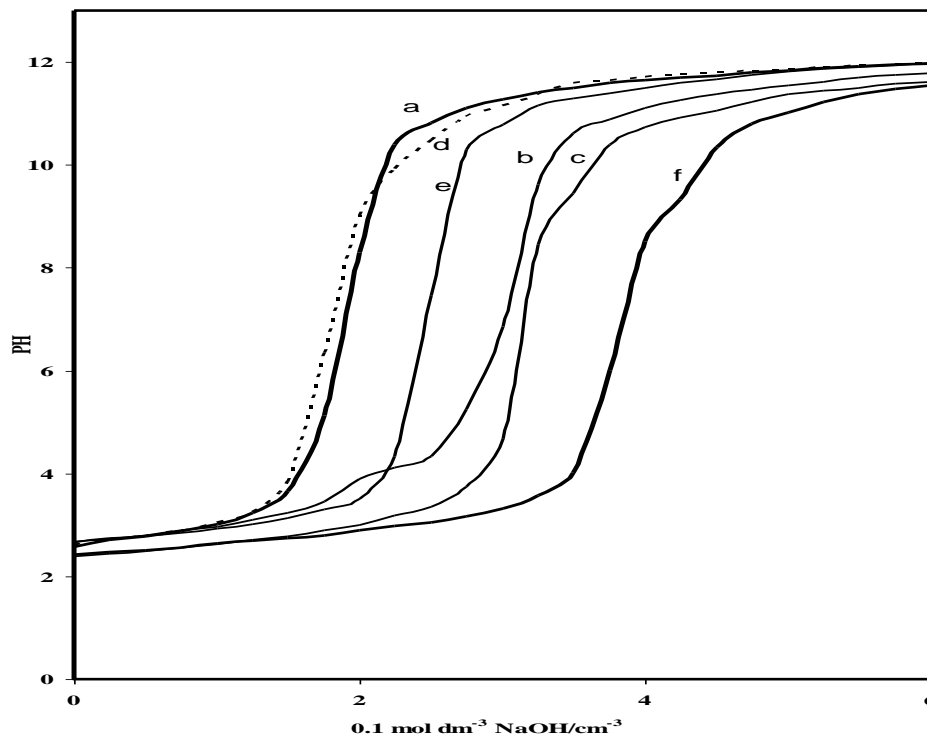
Figure-3
Potentiometric titration curves for the Co(II) + NTA + aspartic acid system at 30 ºC and *I* = 0.1 mol dm^{-3} NaNO$_3$: 0.004 mol dm^{-3} HNO$_3$ + 0.1 mol dm^{-3} NaNO$_3$ (a), solution a + 0.001 mol dm^{-3} NTA (b), solution b + 0.001 mol dm^{-3} Co(II) ion (c), solution a + 0.001 mol dm^{-3} aspartic acid (d), solution d + 0.001 mol dm^{-3} Co(II) ion (e), and solution a + 0.001 mol dm^{-3} Co(II) ion + 0.001 mol dm^{-3} NTA + 0.001 mol dm^{-3} aspartic acid (f)
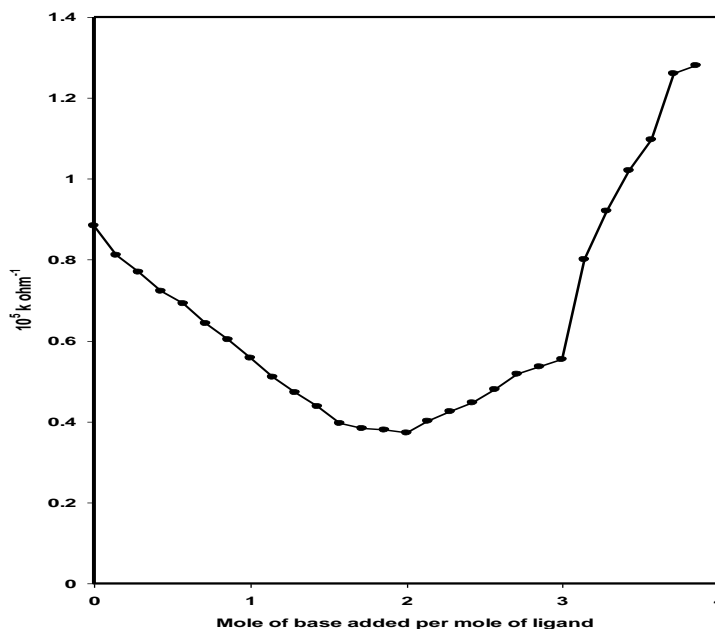
Figure-4
Conductometric titration curve for Co(II)-NTA-phthalic acid systems at 30 ºC and *I* = 0.1 mol·dm^{-3} NaNO$_3$, 0.01 mol dm^{-3} Co(II) + 0.01 mol dm^{-3} NTA + 0.01 mol dm^{-3} phthalic acid
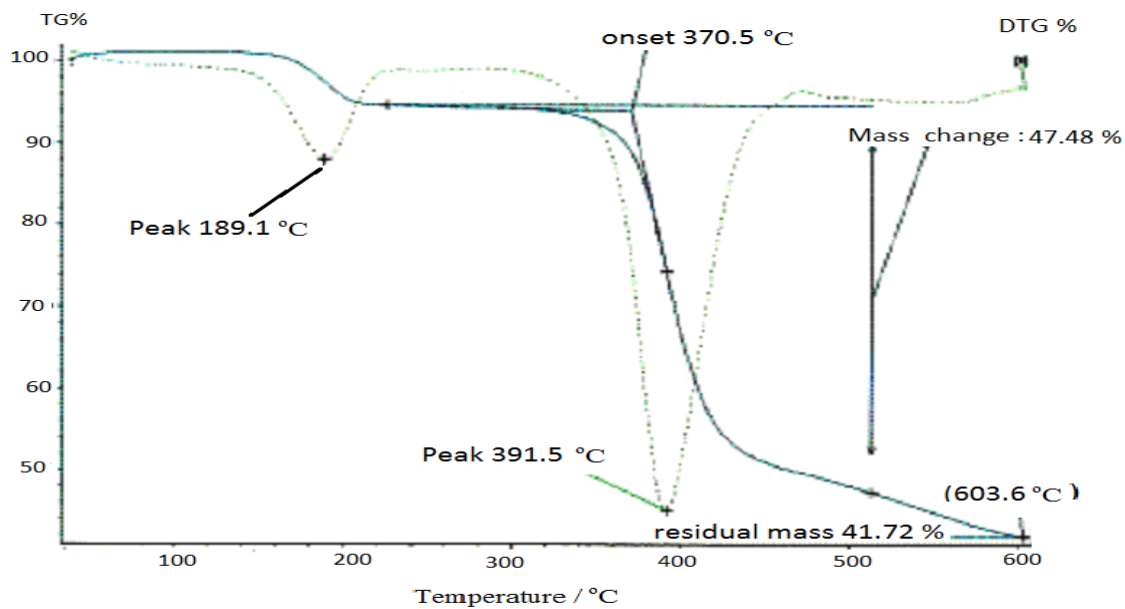
Figure-5
Thermogravimeteric analysis for $[Co^{II}(NTA)(P)(H_2O)]^{3-}$
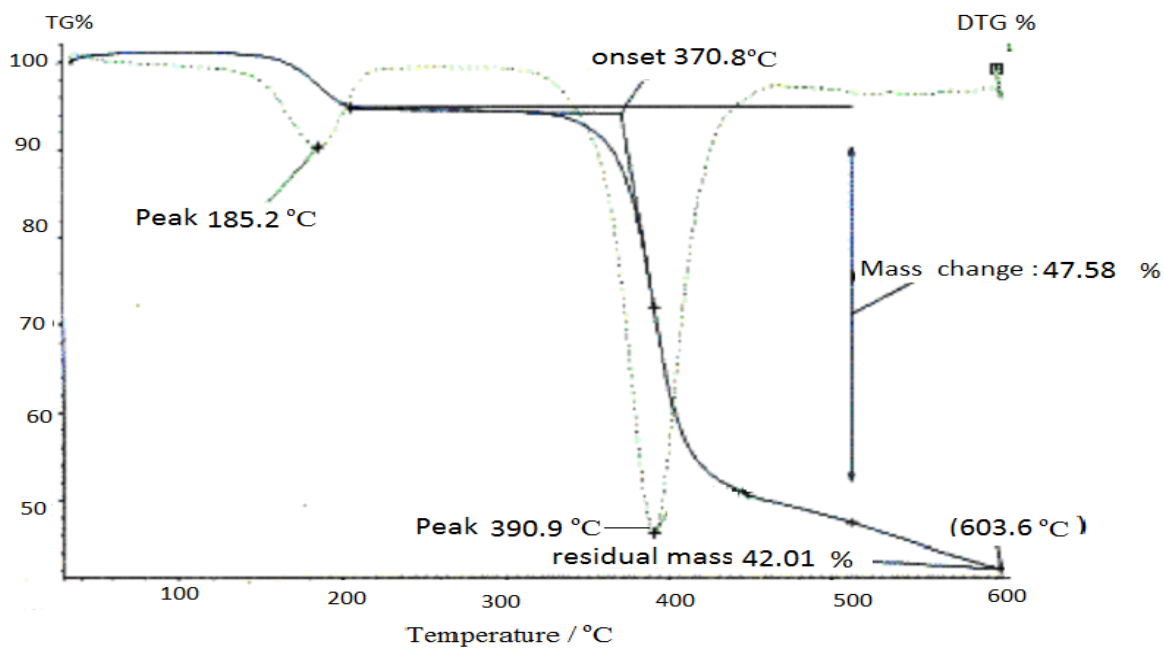
Figure-6
Thermogravimeteric analysis for $[Co^{II}(NTA)(B)(H_2O)_2]^{2-}$

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