RESULTS AND DISCUSSION

3.1. Conductimetric Measurements

3.1.1. Stoichiometric ratio

Conductimetric titrations of the investigated drugs using inorganic complexes, (X^{n-}) were performed to give an insight to the stoichiometric composition of the ion-pairs formed in solutions. The specific conductance values were determined experimentally after each addition, corrected for volume changes, plotted versus the volume added of the titrant and the [drug] / [X^{n-}] ratio was determined. The resulting plots have similar pattern composed of two straight lines intersecting at the end point. The slopes of the branches of the curves are changed widely with species present. These slopes are governed by both the changes in the total number of the electrical charge carried by the ions in the solution and also by the change in the ionic conductance of the ions present.

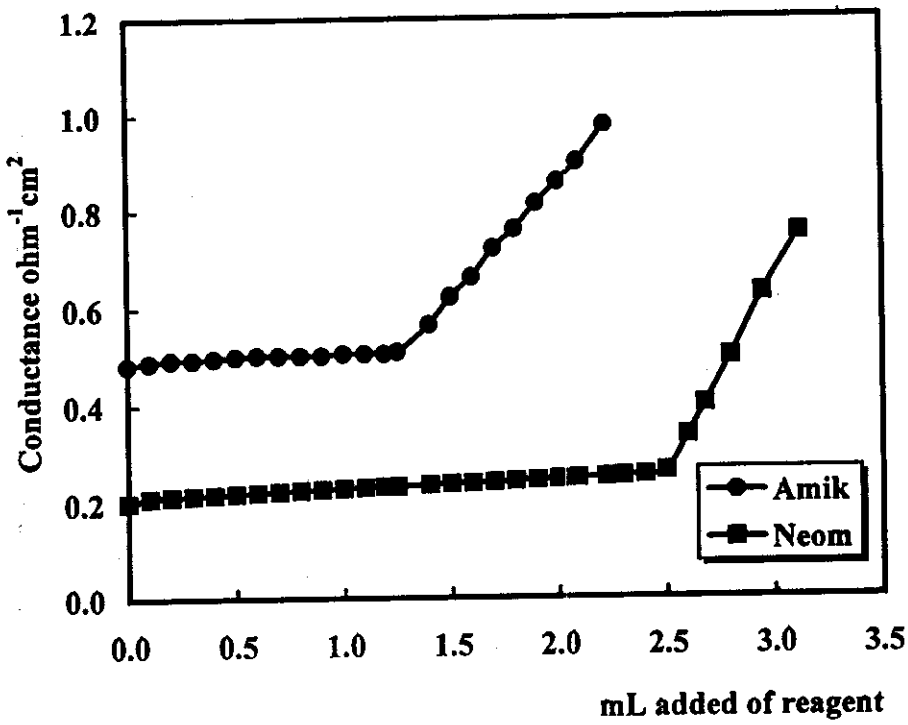
For ammonium reineckate ion-pairs, the characteristic curves break at a volume corresponding to a molecular ratio [drug] / [X^{n-}] = 1/1 for Vanc.Cl, 1/2 for Neom.SO_4, 1/4 for Amik.SO_4 and 1/10 for Tobr.SO_4, confirms the formation of 1:1, 1:2, 1:4 and 1:10 [Drug: X^{n-}] ion-pairs, respectively [Fig. (1)]. For potassium ferrocyanide the two stright lines intersecting at a volume corresponding to 1:1, 2:1, 4:1 and 2:5 molecular ratio for Amik.SO_4, Neom.SO_4, Vanc.Cl and Tobr.SO_4, respectively, [Fig. (2)]. In case of cobalt thiocyanate, nickel thiocyanate and sodium nitroprusside, the plot exhibits a

sharp break at a volume corresponding to 1:1, 1:2, 1:5 and 2:1 molecular ratio for Neom.SO_4, Amik.SO_4, Tobr.SO_4 and Vanc.Cl, respectively, [Fig. (3-5)]. The results obtained coincide fairly with that of the elemental analysis of the solid ion-pairs.

3.1.2. Determination of the investigated drugs in pure forms, pharmaceutical preparations and in urine samples using conductimetric titration

Electrolytes when dissolved in water dissociate into ions; which are capable of transporting electricity and thus the solution becomes conducting to electric current. Different kind of ions have different velocities, so that when an electric current is passed through a solution, the faster moving ions carry a relatively great amount of the current. The conductance of a solution, which is a measure of the mobility of different ions, is the reciprocal of its electrical resistance.

Conductance measurement can be used, successfully for the equivalent point determination in titration of systems in which the conductance of the solution varies before and after the end point. Many precipitation titrations are possible by conductimetric method. In this case, the formation of a precipitate alter the number of ions present in the solution and so the conductance varies. After the equivalent point, the addition of excess titrant increase the number of ions and so the conductance increases. The titration curve, which is a relation between the conductance and the volume of the titrant added, can be represented by two lines intersecting at the end-point.



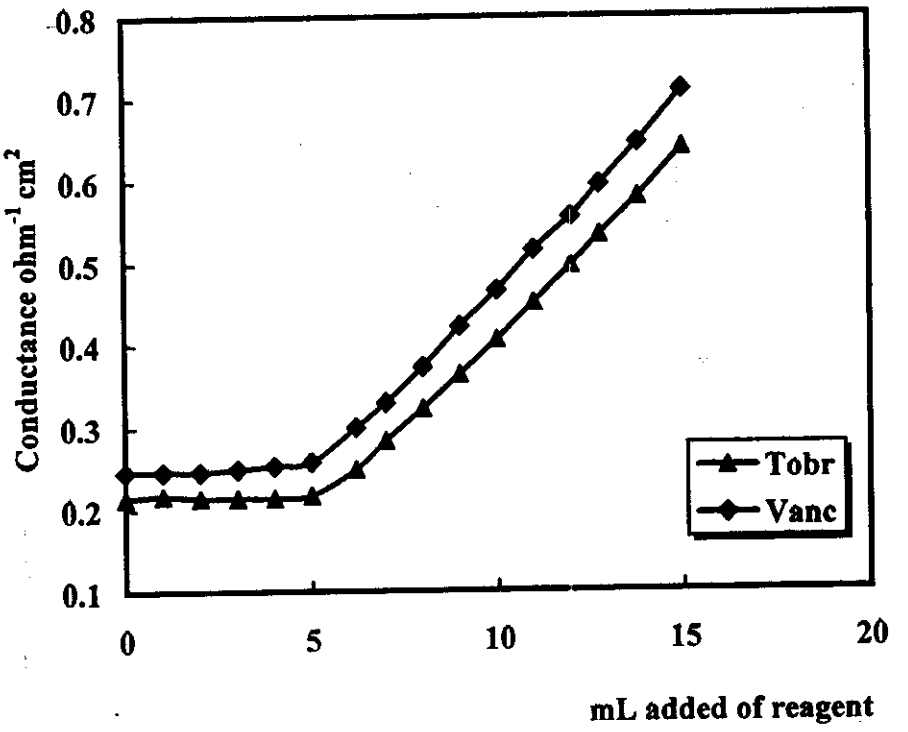
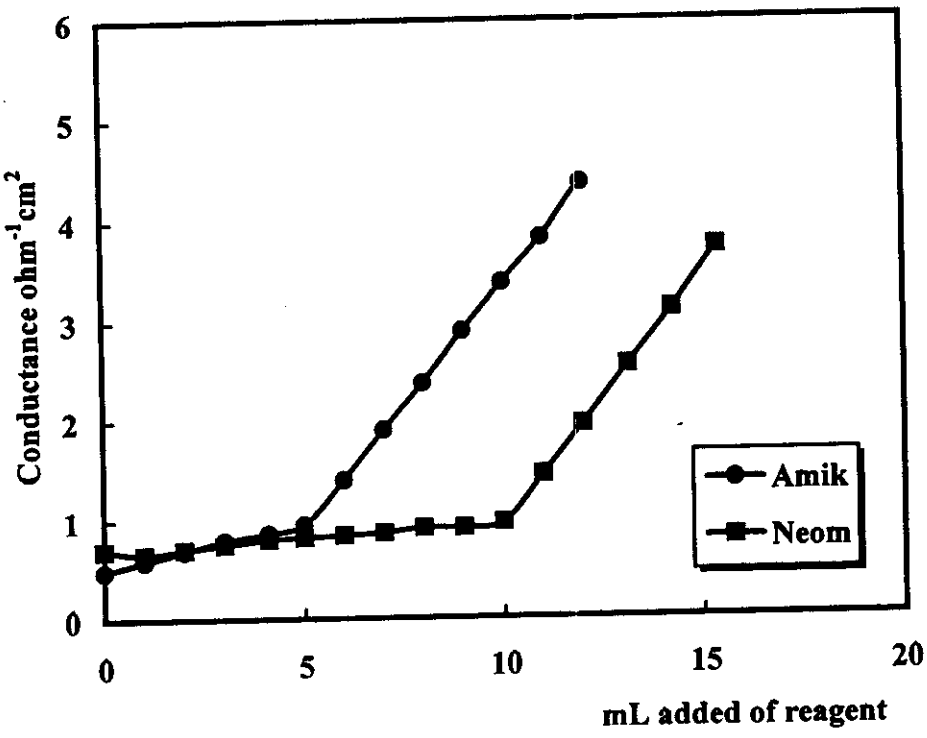


Fig. (1): Conductimetric titration of 5 x 10^{-5} M Amik.SO_4, Neom.SO_4 and Vanc.Cl using 5 x 10^{-4} M $NH_4[Cr(NH_3)_2(SCN)_4]$ and 5 x 10^{-5} M reagent for Tobr.SO_4



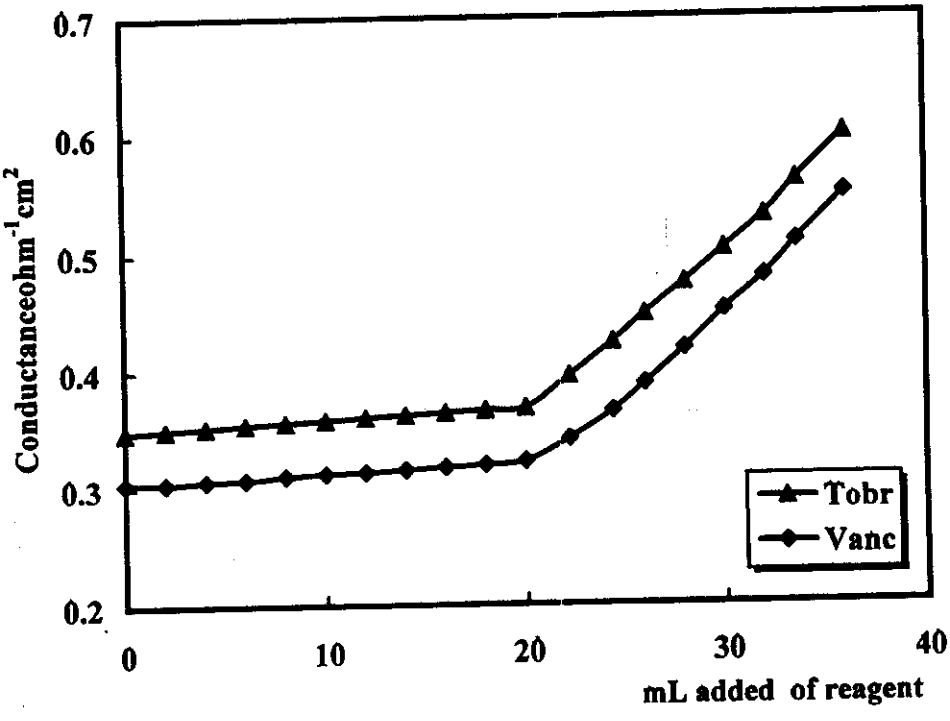
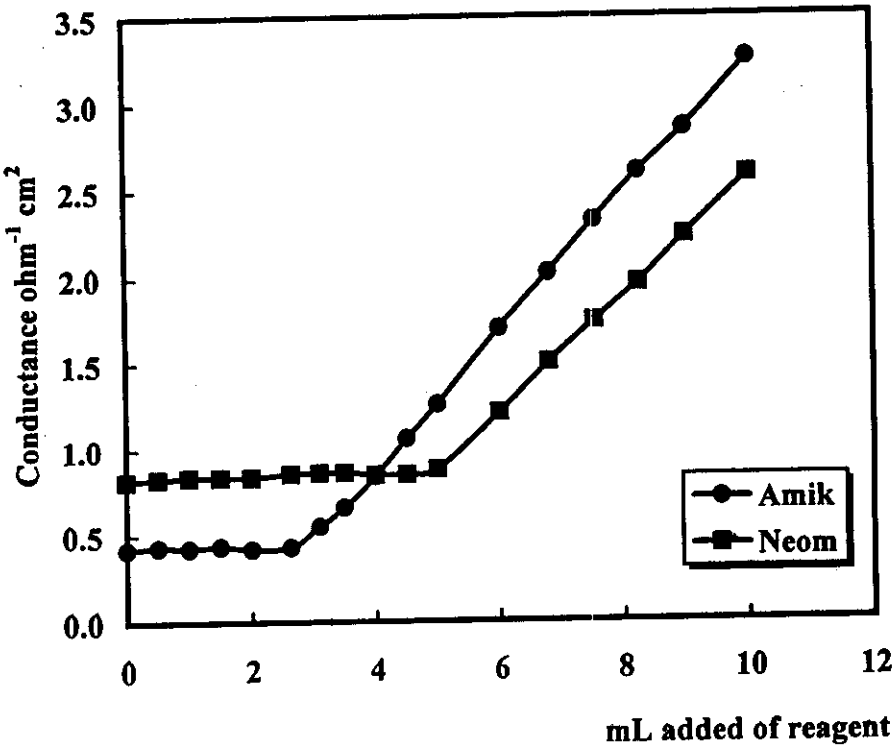


Fig. (2): Conductimetric titration of 5 x 10^{-5} M Amik.SO_4, Neom.SO_4 and Vanc.Cl using 5 x 10^{-4} M $K_4[Fe(CN)_6]$ and 5 x 10^{-5} M reagent for Tobr.SO_4



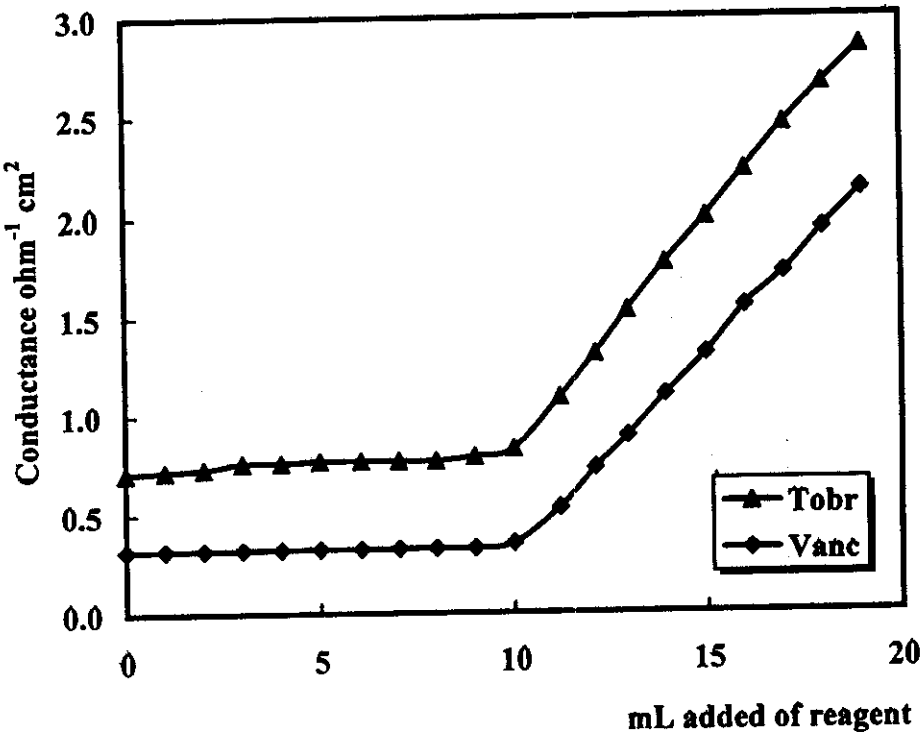


Fig. (3): Conductimetric titration of 5 x 10^{-5} M Amik.SO_4, Neom.SO_4 and Vanc.Cl using 5 x 10^{-4} M $(NH_4)_2[Co(SCN)_4]$ and 5 x 10^{-5} M reagent for Tobr.SO_4

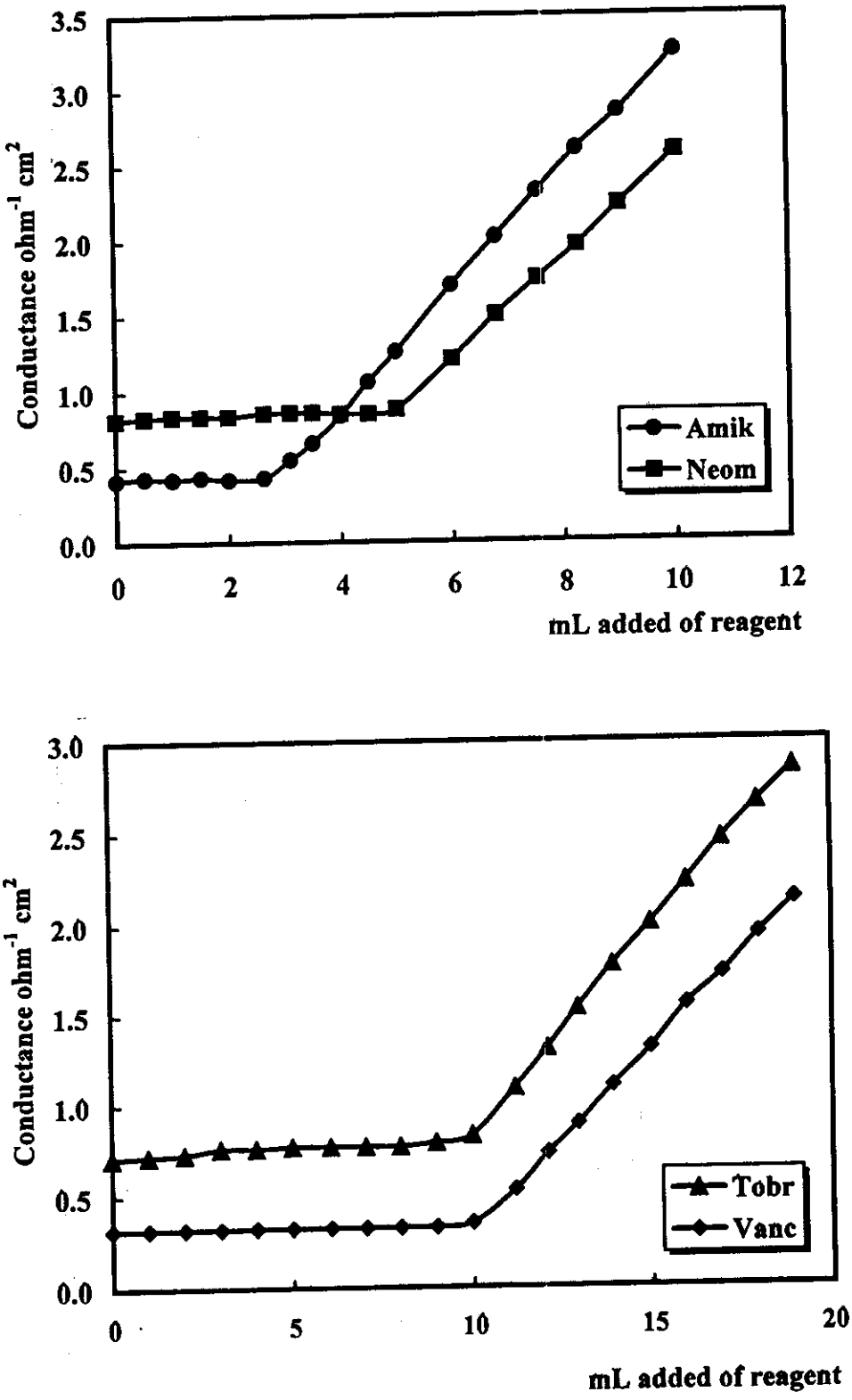


Fig. (4): Conductimetric titration of 5 x 10^{-5} M Amik.SO_4, Neom.SO_4 and Vanc.Cl using 5 x 10^{-4} M $(NH_4)_2[Ni(SCN)_4]$ and 5 x 10^{-5} M reagent for Tobr.SO_4

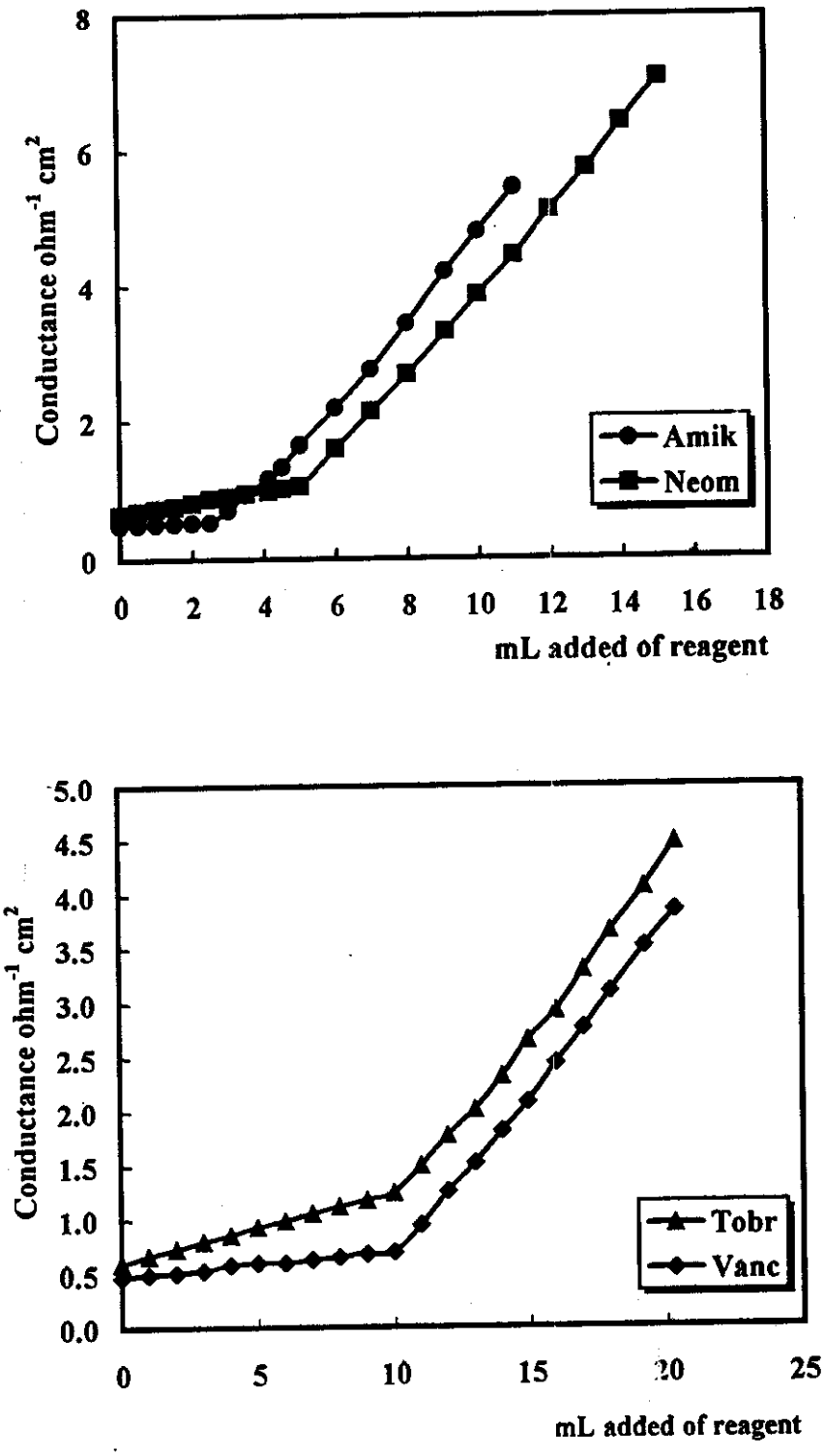


Fig. (5): Conductimetric titration of 5 x 10^{-5} M Amik.SO_4, Neom.SO_4 and Vanc.Cl using 5 x 10^{-4} M $Na_2[Fe(CN)_5NO]$ and 5 x 10^{-5} M reagent for Tobr.SO_4.

Since all the studied drugs are present in the sulphate and hydrochloride forms and they able to form a precipitate with inorganic complex ions, so the applicability of conductimetric titration of these drugs with some inorganic complexes ion was tested. The different variables affecting the conductimetric titration curve were studied.

3.1.3. Factors affecting the end point of conductimetric titration of the investigated drugs with ammonium reineckate, potassium ferrocyanide, cobalt thiocyanate, nickel thiocyanate and sodium nitroprusside

3.1.3.1. Effect of solvent

Titration of Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl against ammonium reineckate, potassium ferrocyanide, cobalt thiocyanate, nickel thiocyanate and sodium nitroprusside in different media (aqueous solution, up to 50% ethanol-water, methanol- water, propanol- water, acetone-water and dioxan-water) was carried out to establish the best conditions for their conductimetric determination. The results obtained in all media, except aqueous medium, showed that the shape of the titration curves is not suitable for their conductimetric titration as the conductance measurements are scattered, non-linearly around the end point. The aqueous media was found to be the most suitable media for conductimetric titration of Amik.SO_4, Neom.SO_4, Tob.SO_4 and Vanc.Cl using ammonium reineckate, potassium ferrocyanide, cobalt thiocyanate, nickel thiocyanate and sodium nitroprusside. In all cases, the titration

curves consists of two straight lines intersecting at the end point. So that, the best media to achieve sharp inflection was the aqueous media.

3.1.3.2. Effect of temperature

The effect of temperature on the evaluation of the end point in conductimetric titration of 1.1725, 1.3635, 0.7125 and 0.7425 mg of Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl, respectively, using $5x10^{-5}$M ammonium reineckate (in case of Tobr.SO_4) and $5x10^{-4}$M (for the other drugs). 0.3910, 0.4545, 0.3560 and 0.3710 mg of Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl, respectively using $5x10^{-5}$ M potassium ferrocyanide (in case of Tobr.SO_4) and $5x10^{-4}$ M (for the other drugs). 1.9545, 2.2725, 2.1380 and 2.2285 mg of Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl, respectively, using $5x10^{-5}$ M of cobalt thiocyanate, nickel thiocyanate and sodium nitroprusside (in case of Tobr.SO_4) and $5x10^{-4}$ M (for other drugs), was studied by carrying out the titration at 25, 30, 40 and 50 °C. From the obtained results, it was found that as the temperature increase, the conductivity of the whole solution increase and no effect is observed on the shape of the titration curves and the position of the end point. All further works were carried out at ambient temperature (25 ± 1°C).

3.1.3. 3. Effect of the dilution of titrand and titrant

The effect of dilution of the titrand and the titrant on the end point of conductimetric titration of the investigated drugs were also studied

a- Titrand effect

A certain amount of the investigated drugs 1.1725, 1.3635, 0.7125 and 0.7425 mg of Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl, respectively, in case of ammonium reineckate, 0.3910, 0.4545, 0.3560 and 0.3710 mg of Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl, respectively, in case of potassium ferrocyanide or 1.9545, 2.2725, 2.1380 and 2.2285 mg of Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl, respectively, in case of cobalt thiocyanate, nickel thiocyanate or sodium nitroprusside, was diluted to 50, 75 and 100 mL with distilled water then titrated with the studied reagent using $5x10^{-5}$ M for Tobr.SO_4 and $5x10^{-4}$ M for other studied drugs. The results showed that, dilution of the titrand up to 100 mL has no effect on the position of the end point and the shape of the titration curve. The optimum volume to achieve sharp inflection was 50 mL.

b- Titrant effect

A certain amount of the investigated drugs 1.1725, 1.3635, 0.7125 and 0.7425 mg of Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl, respectively, in case of ammonium reineckate and 0.3910, 0.4545, 0.3560 and 0.3710 mg of Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl, respectively, in case of potassium ferrocyanide or 1.9545, 2.2725, 2.1380 and 2.2285 mg of Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl, respectively, in case of cobalt thiocyanate, nickel thiocyanate and sodium nitroprusside was diluted to 50 mL and titrated against $5x10^{-6}$, $8x10^{-6}$, $1x10^{-5}$, $3x10^{-5}$, $5x10^{-5}$, $7x10^{-5}$, $1x10^{-4}$, $5x10^{-4}$ and $7x10^{-4}$ M solutions of ammonium reineckate or potassium ferrocyanide, cobalt thiocyanate, nickel thiocyanate and

sodium nitroprusside. The results indicated that, titrant solutions lower than $5x10^{-4}$ M are not suitable for conductimetric titrations of Amik.SO_4, Neom.SO_4 and Vanc.Cl, whereas titrant solutions lower than 5 x 10^{-5} M are not suitable for conductimetric titrations of Tobr.SO_4 as the conductance reading are unstable. Also, a very poor inflection at the end point was observed.

The results obtained indicated that, the conductimetric titration of Amik.SO_4, Neom.SO_4 and Vanc.Cl against $5x10^{-4}$ M ammonium reineckate, potassium ferrocyanide, cobalt thiocyanate, nickel thiocyanate or sodium nitroprusside can be successfully performed in aqueous medium at ambient temperature up to 50 °C, whereas in case of Tobr.SO_4, the titration occur with $5x10^{-5}$ using the same reagents.

3.1.4. Conductimetric determination of Amik.SO_4, Neom.SO_4, Tob.SO_4 and Vanc.Cl in pure forms

Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl have been determined in pure solution using the following procedure:

Aliquot containing 0.1563-3.5178, 0.1363-4.0900, 0.0712-6.4156 and 0.0742-2.2285 mg of Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl, respectively, using ammonium reinckate, 0.0390-3.5179, 0.0454-4.0900, 0.0712-3.5642 and 0.0297-2.2285 mg of Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl, respectively, using potassium ferrocyanide or 0.0781-3.5178, 0.0454-4.0900, 0.0712-3.5642 and 0.0594-2.2285 mg of Amik.SO_4, Neom.SO_4, Tobr.SO_4

and Vanc.Cl, respectively, using cobalt thiocyanate, nickel thiocyanate or sodium nitroprusside was transferred to a 50 mL calibrated flask, then completed up to the mark with bidistilled water. The contents of the calibrated flask were transferred to a titration cell and the conductivity cell was immersed in. The reagents solutions were added from a microburette and the conductance was measured subsequent to each addition of the reagent solutions, after through stirring, at room temperature (25 ± 1 °C). The conductance reading take 1-2 min after each addition was corrected for dilution.

A graph of corrected conductivity versus the volume added of the titrant was constructed and the end point was determined as illustrated in [Figs. (6-25)]. 0.1 mL of $5x10^{-4}$ ammonium reineckate is equivalent to 3.120, 1.818 and 1.486 µg of Amik.SO_4, Neom.SO_4 and Vanc.Cl, respectively, whereas 0.1 mL of $5x10^{-5}$ M ammonium reineckate is equivalent to 1.425 µg of Tobr.SO_4. 0.1 mL of $5x10^{-4}$ M potassium ferrocyanide is equivalent to 0.782, 0.454 and 0.371 µg of Amik.SO_4, Neom.SO_4 and Vanc.Cl, respectively, whereas 0.1 mL of $5x10^{-5}$ M of this reagent is equivalent to 0.356 µg of Tobr.SO_4. Using cobalt thiocyanate, nickel thiocyanate or sodium nitroprusside, 0.1 mL of $5x10^{-4}$ M is equivalent to 1.560, 0.909 and 0.742 µg of Amik.SO_4, Neom.SO_4, Vanc.Cl, respectively, whereas 0.1 mL of $5x10^{-4}$ M is equivalent to 0.713 µg of Tobr.SO_4.

The results given in [Table (2) and (3)] showed that, the present method are highly precise and accurate as the standard deviations and the recoveries present are within the ranges 0.03-0.09

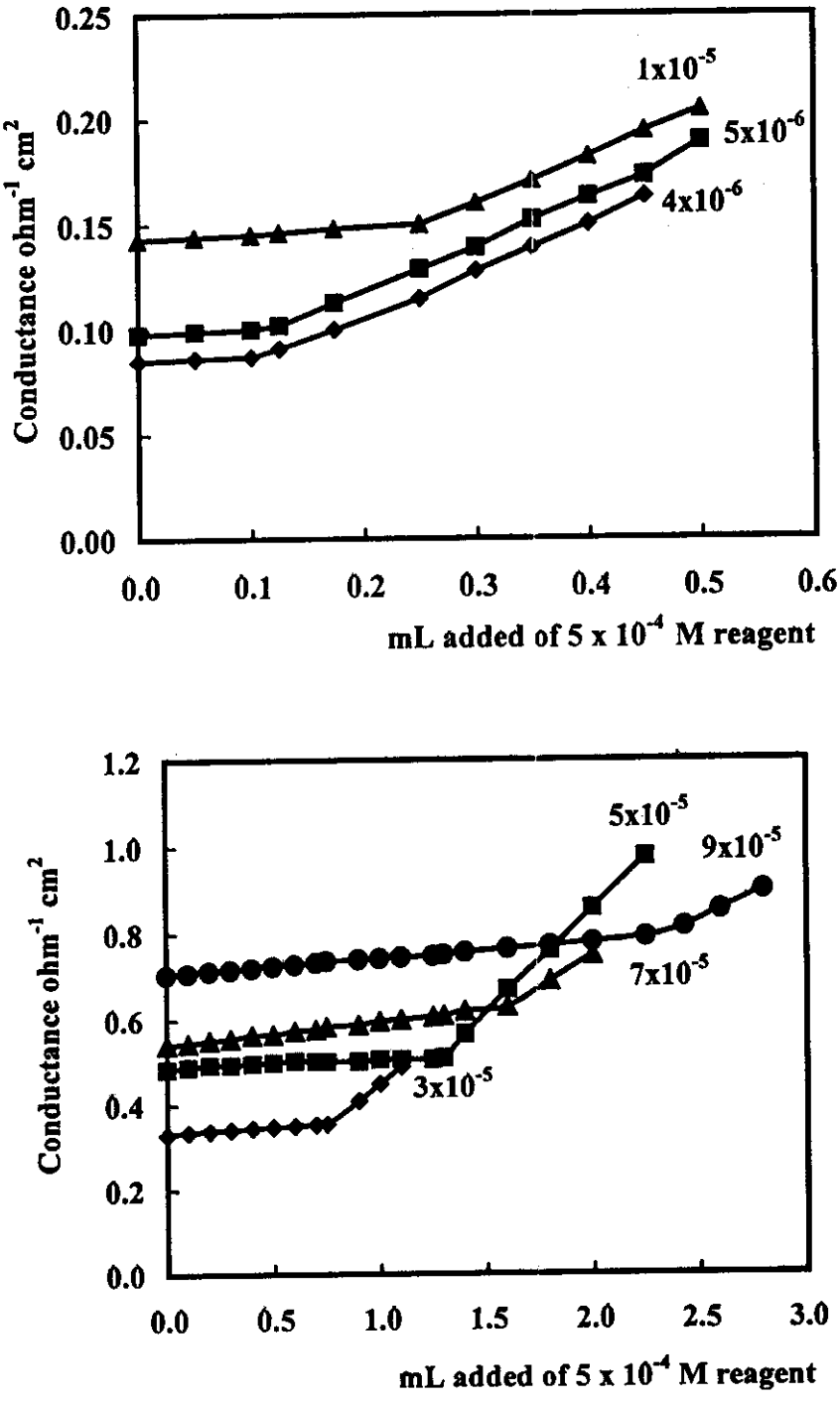
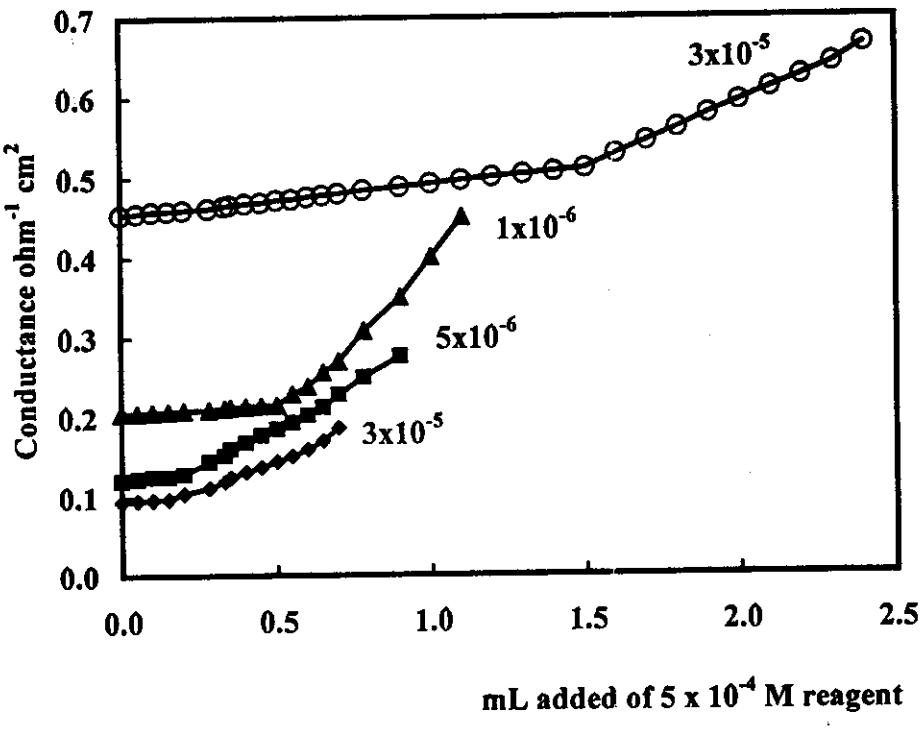


Fig. (6): Conductimetric titration of different concentration of Amik.SO_4 using 5 x 10^{-4} M $NH_4[Cr(NH_3)_2(SCN)_4]$



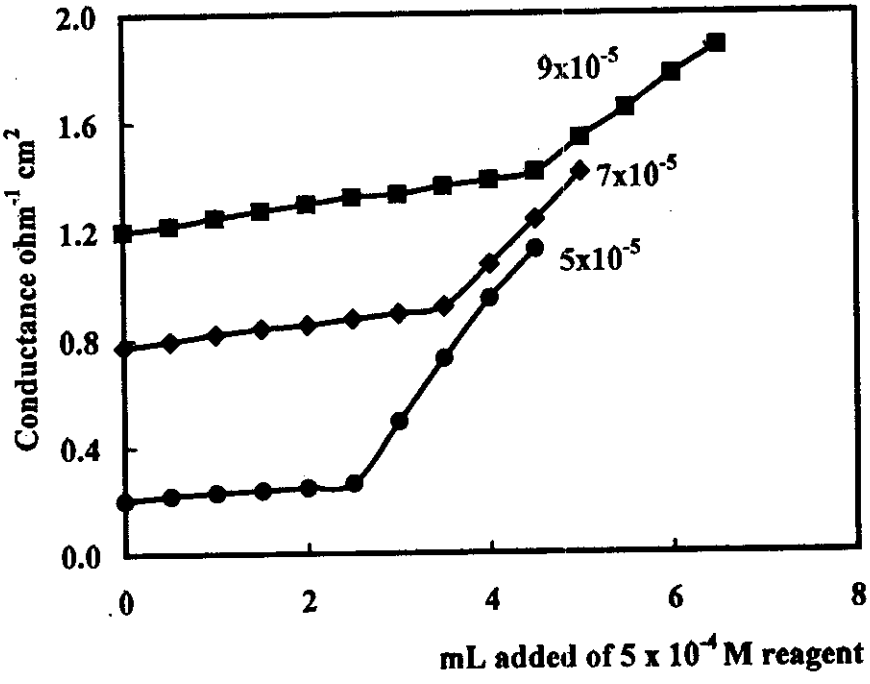
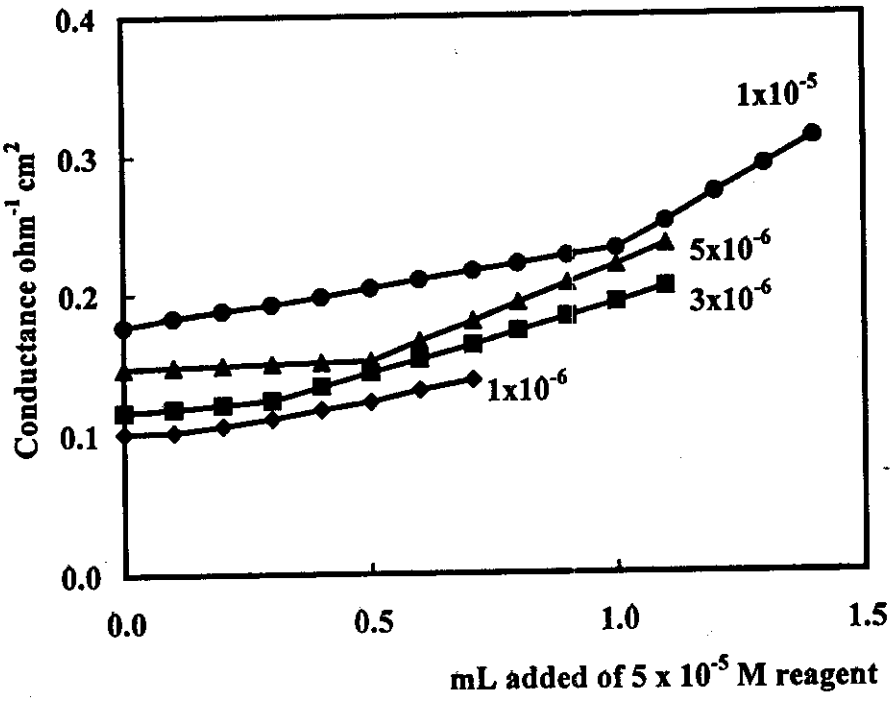


Fig. (7): Conductimetric titration of different concentration of Neom.SO_4 using 5 x 10^{-4} M $NH_4[Cr(NH_3)_2(SCN)_4]$



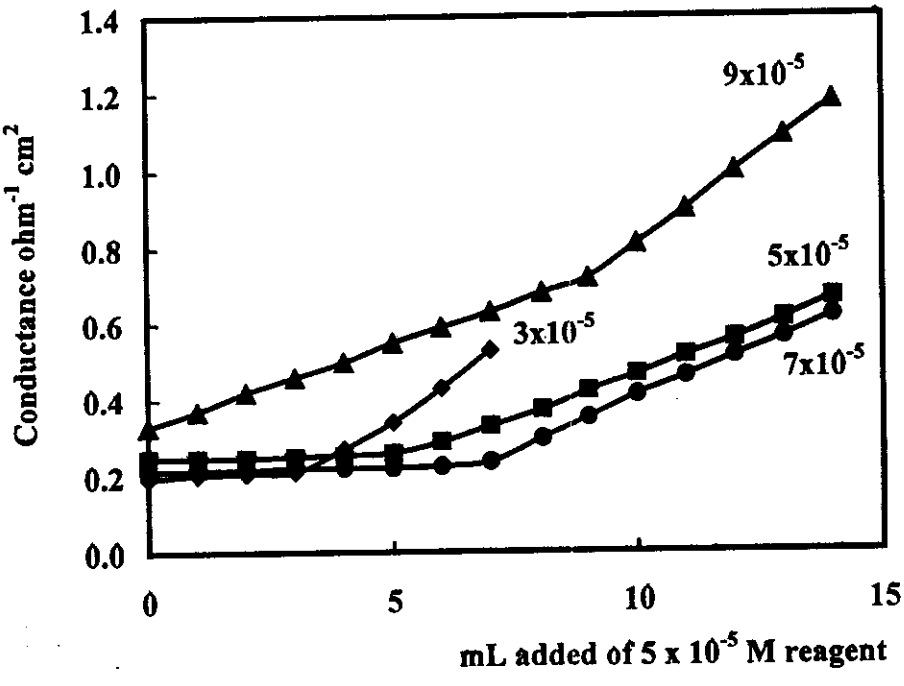
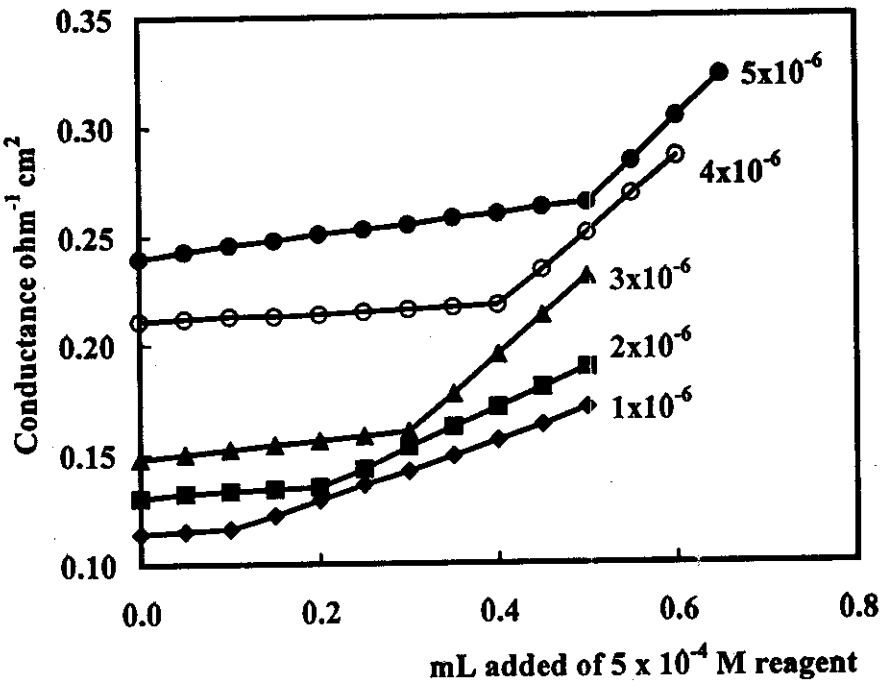


Fig. (8): Conductimetric titration of different concentration of Tobr.SO_4 using 5 x 10^{-5} M $NH_4[Cr(NH_3)_2(SCN)_4]$



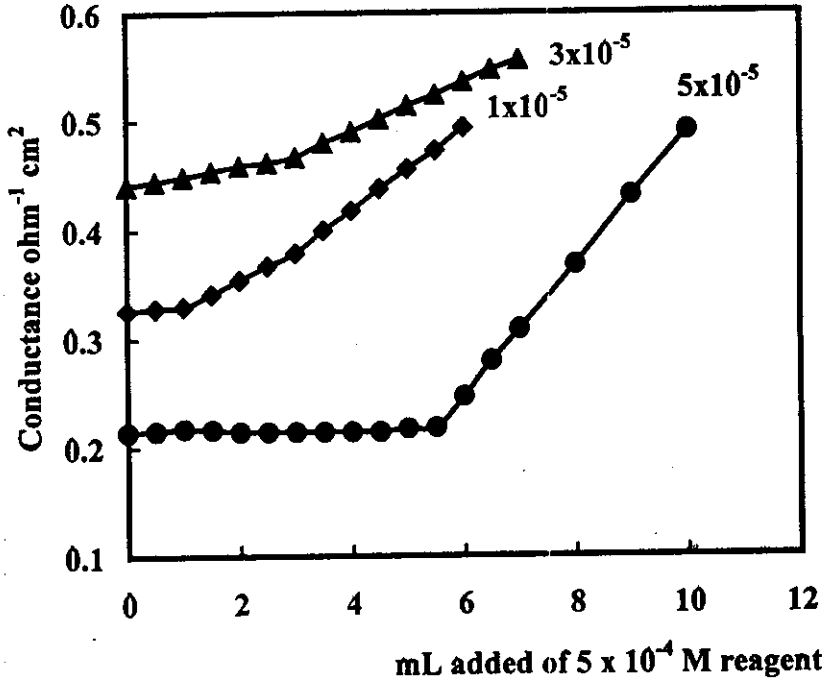
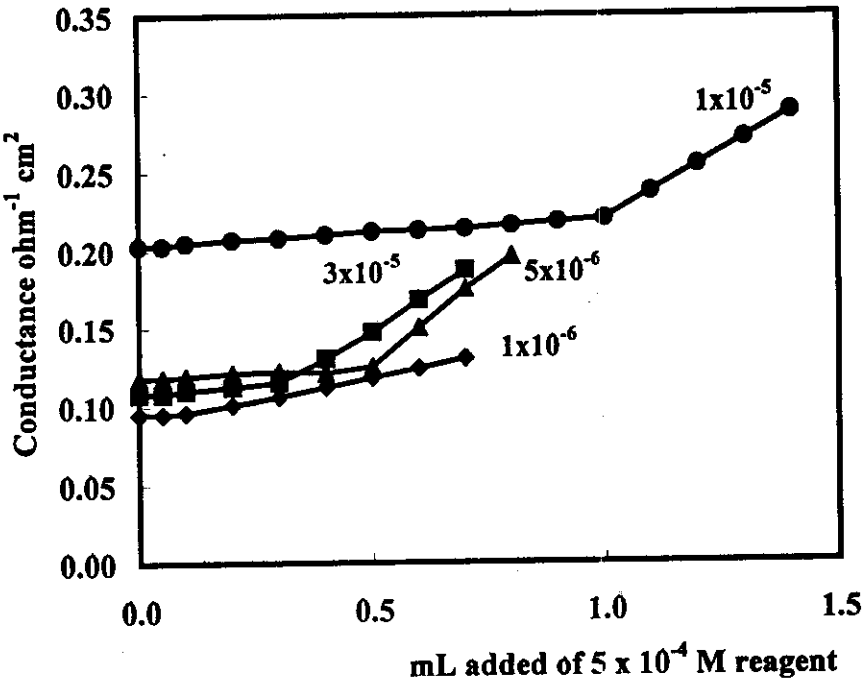


Fig. (9): Conductimetric titration of different concentration of Vanc.Cl using 5 x 10^{-4} M $NH_4[Cr(NH_3)_2(SCN)_4]$



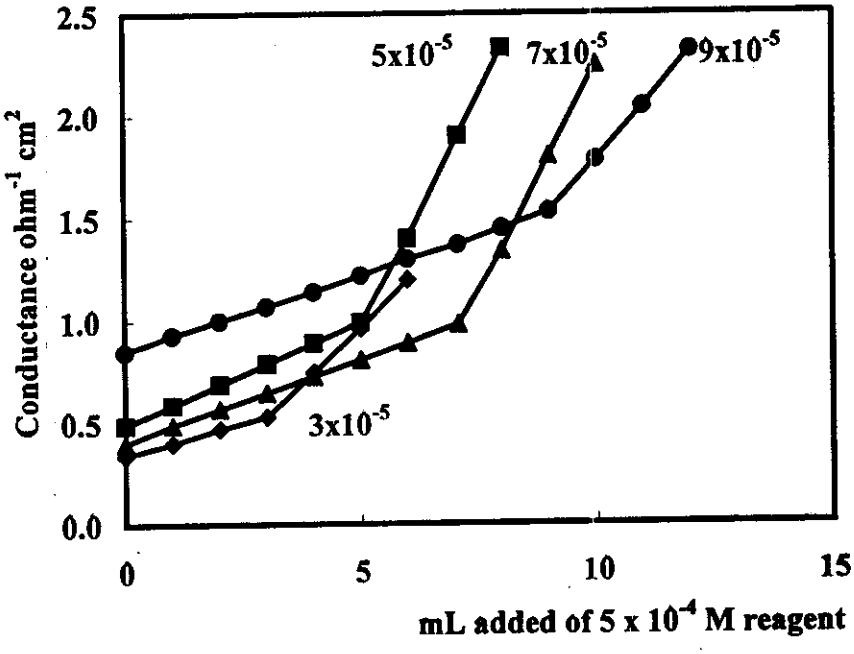


Fig. (10): Conductimetric titration of different concentration of Amik.SO_4 using 5 x 10^{-4} M $K_4[Fe(CN)_6]$

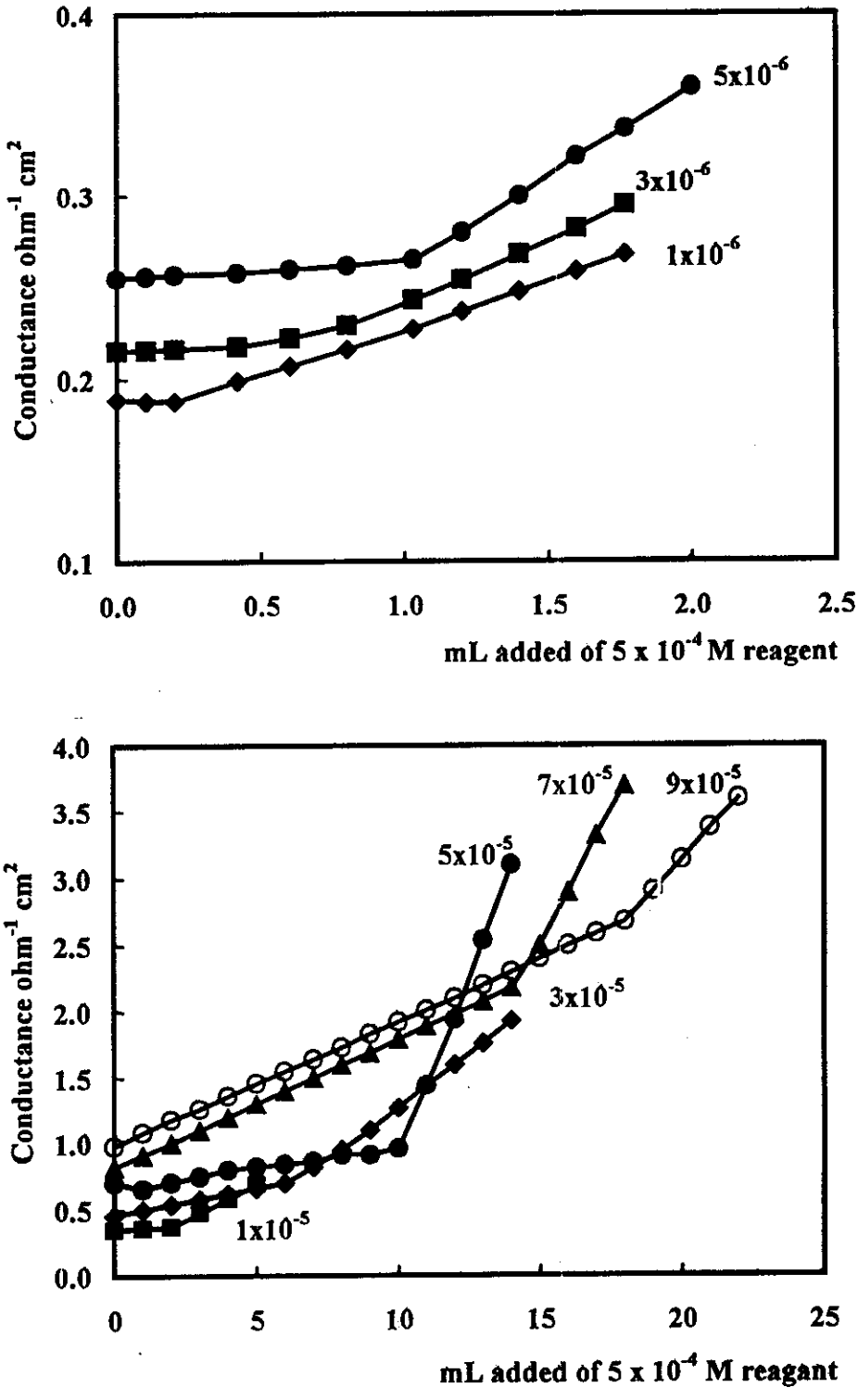


Fig. (11): Conductimetric titration of different concentration of Neom.SO_4 using 5 x 10^{-4} M $K_4[Fe(CN)_6]$

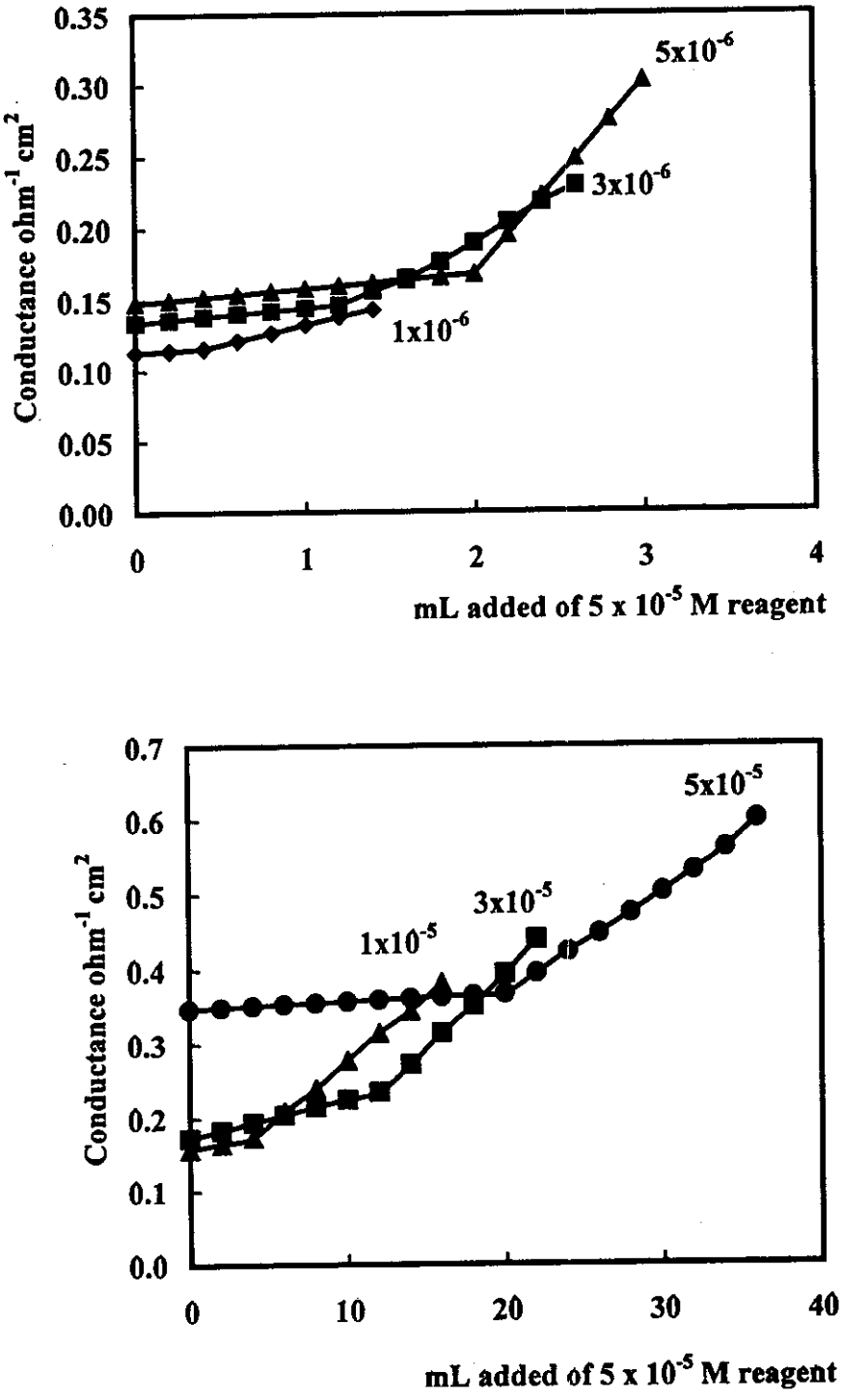


Fig. (12): Conductimetric titration of different concentration of Tobr.SO_4 using 5 x 10^{-5} M $K_4[Fe(CN)_6]$

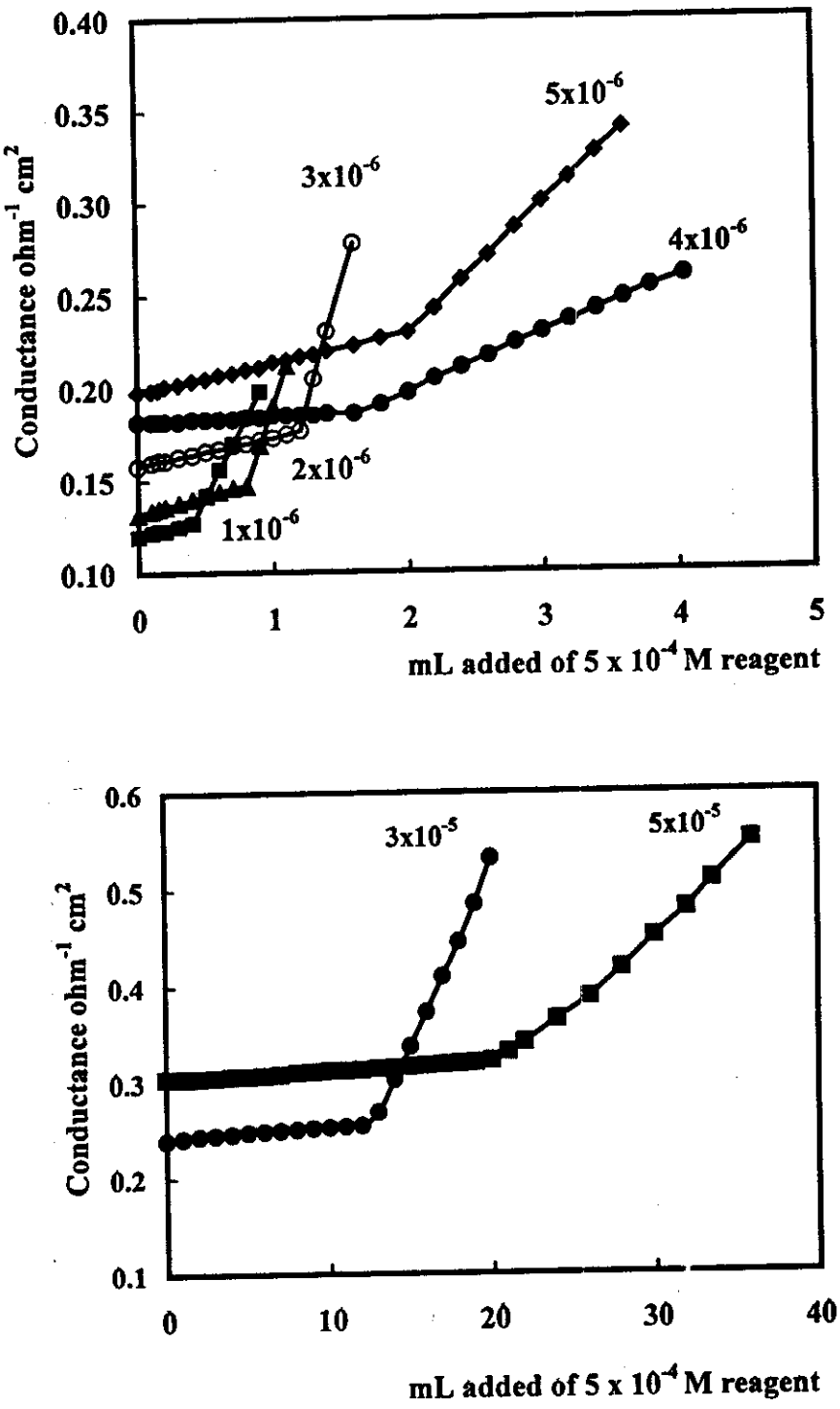
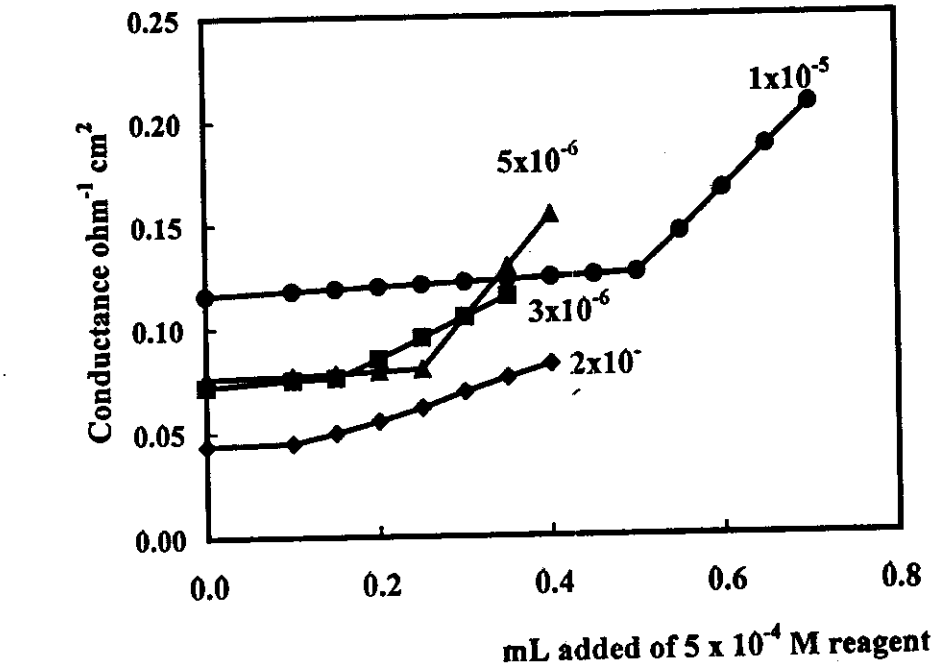


Fig. (13): Conductimetric titration of different cincentration of Vanc.Cl using 5 x 10^{-4} $K_4[Fe(CN)_6]$



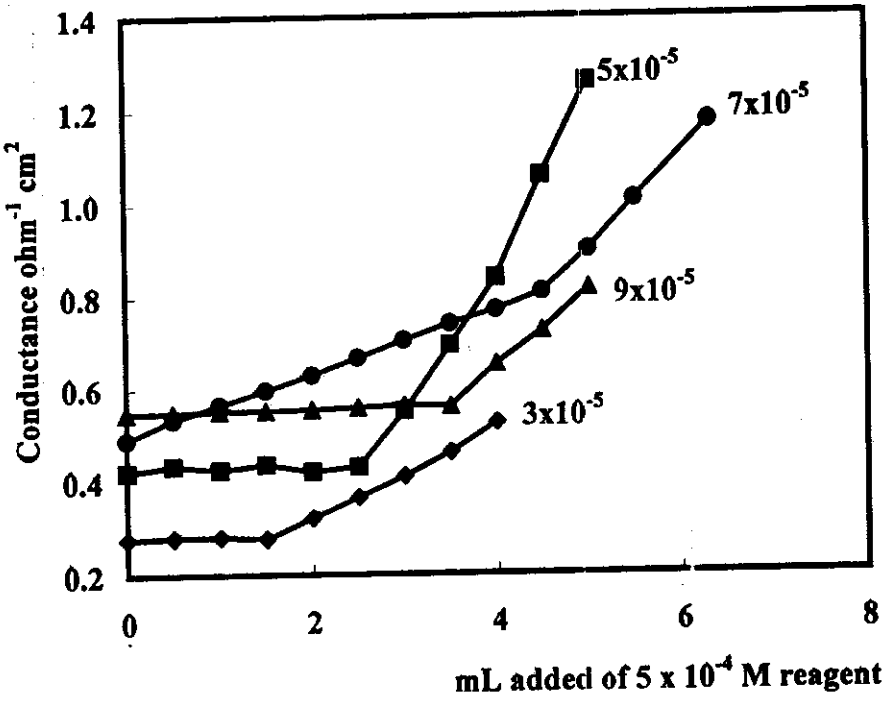
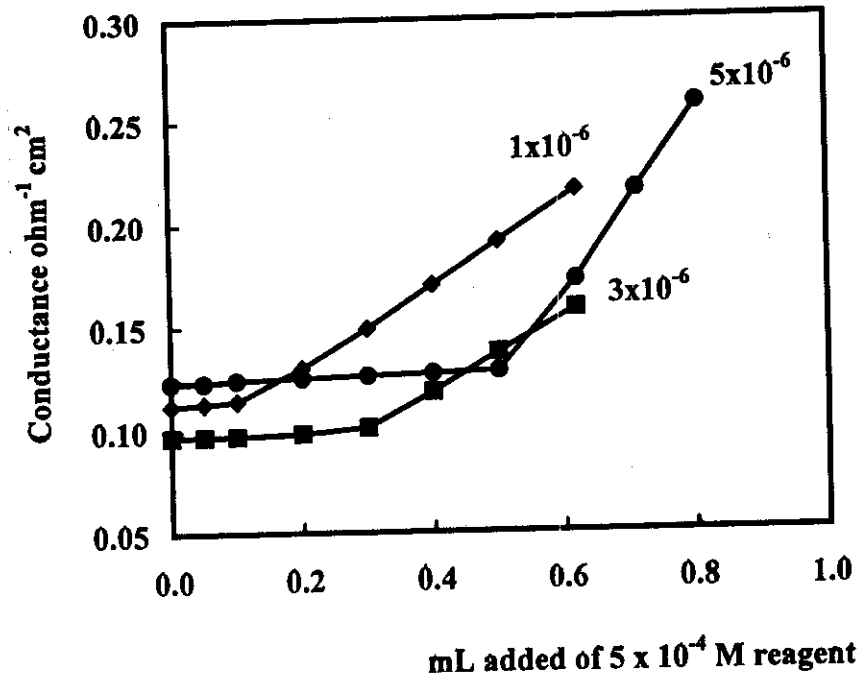


Fig. (14): Conductimetric titration of different concentration of Amik.SO_4 using 5 x 10^{-4} M $(NH_4)_2[Co(SCN)_4]$



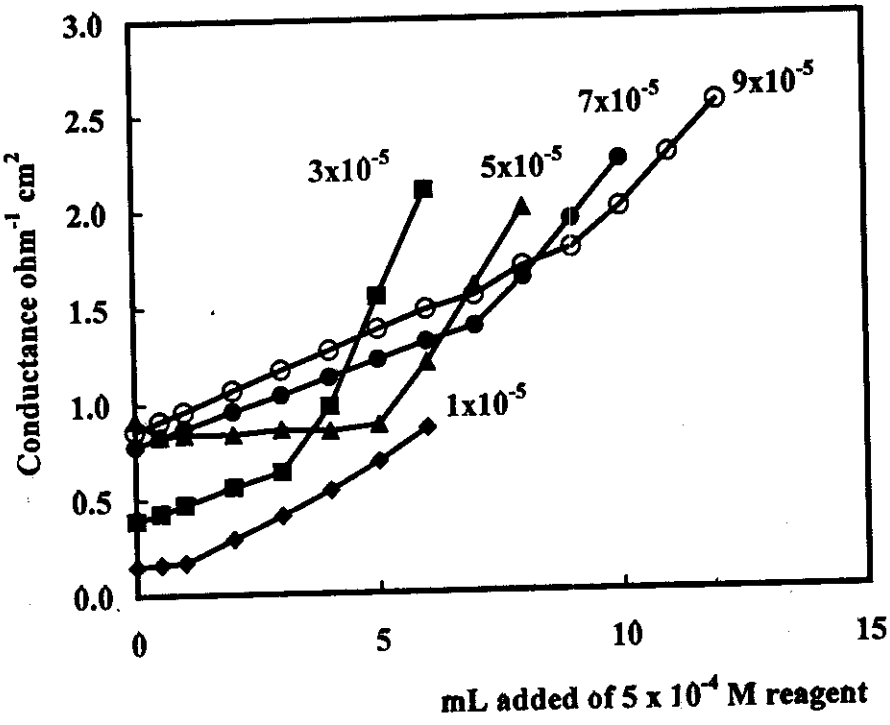
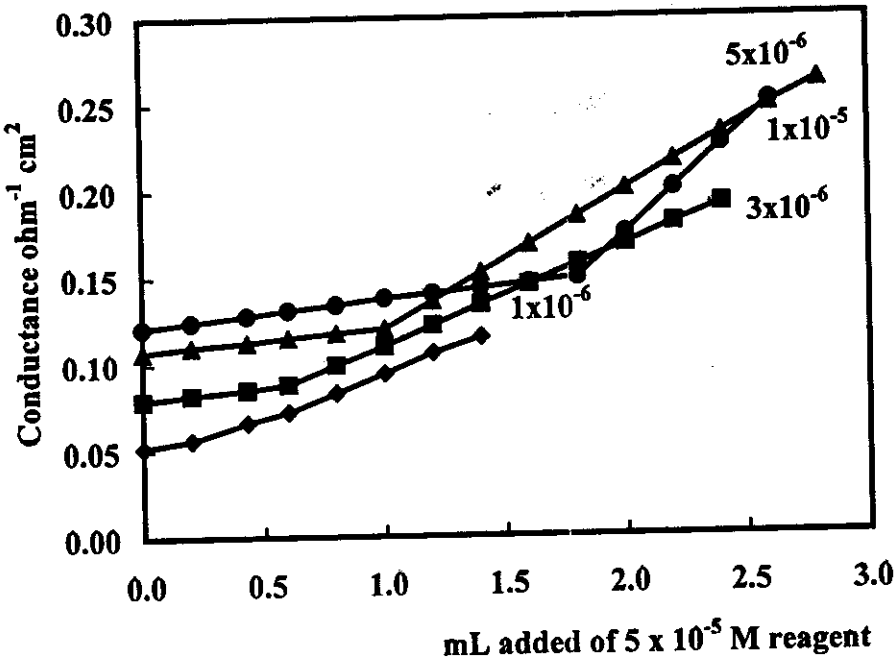


Fig. (15): Conductimetric titration of different concentration of Neom.SO_4 using 5 x 10^{-4} M $(NH_4)_2[Co(SCN)_4]$



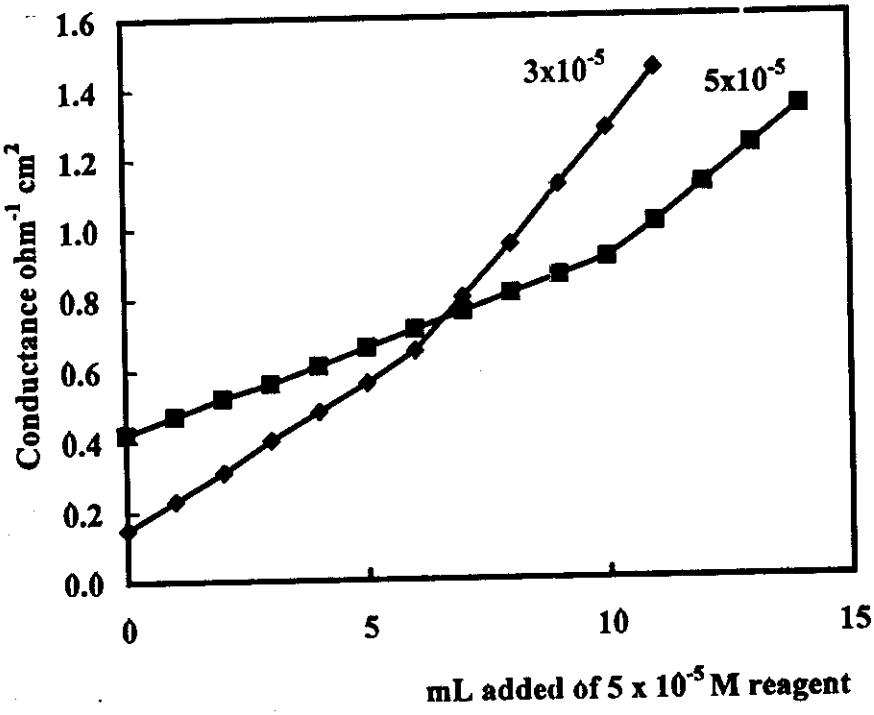


Fig. (16): Conductimetric titration of different concentration of Tobr.SO_4 using 5 x 10^{-5} M $(NH_4)_2[Co(SCN)_4]$

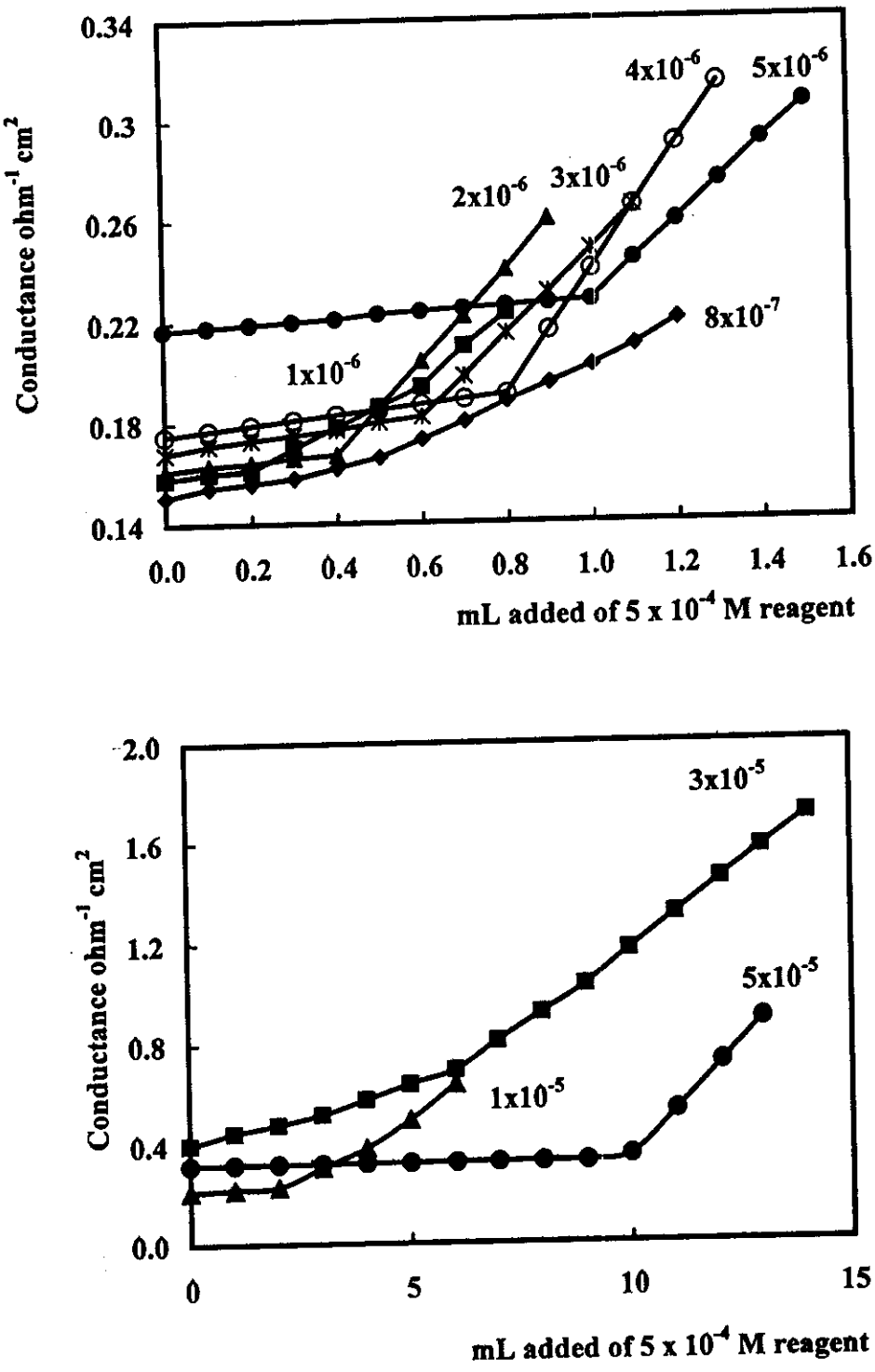
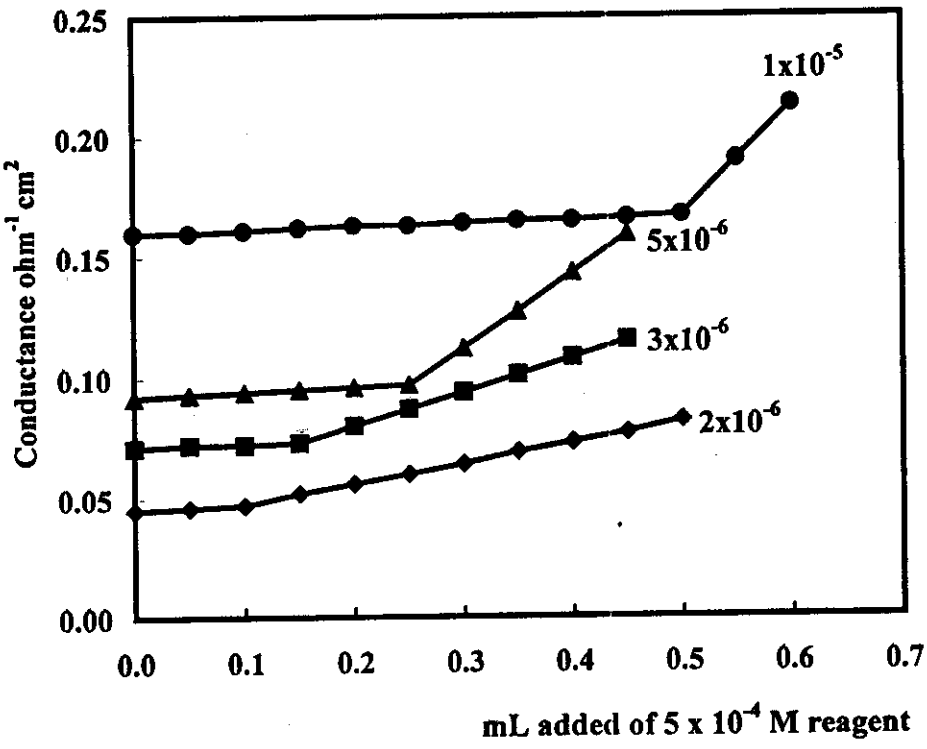


Fig. (17): Conductimetric titration of different concentration of Vanc.Cl using 5 x 10^{-4} M $(NH_4)_2[Co(SCN)_4]$



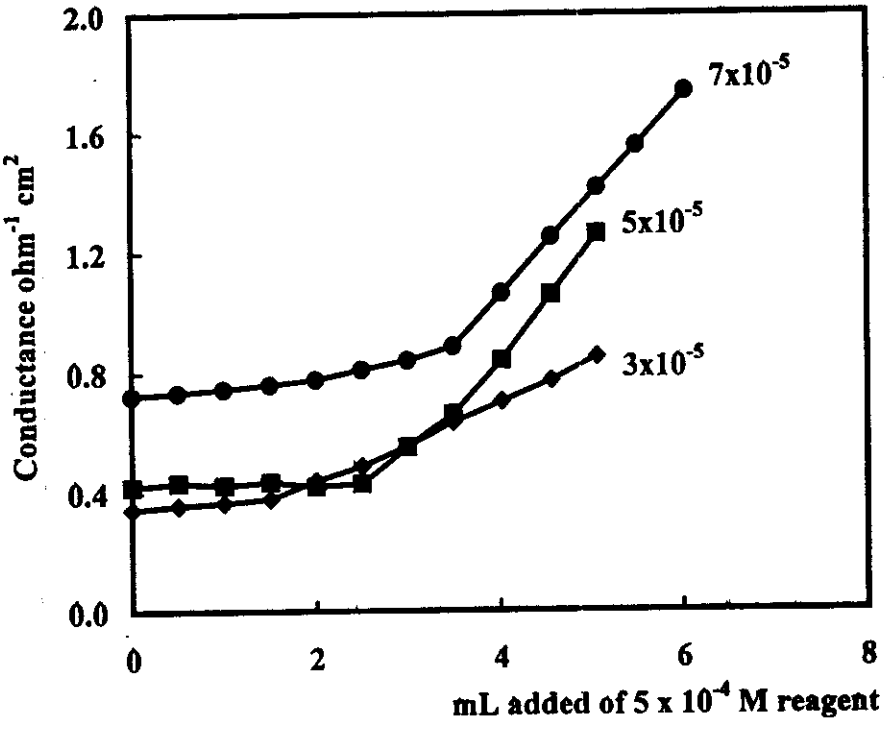


Fig. (18): Conductimetric titration of different concentration of Amik.SO_4 using 5 x 10^{-4} M $(NH_4)_2[Ni(SCN)_4]$

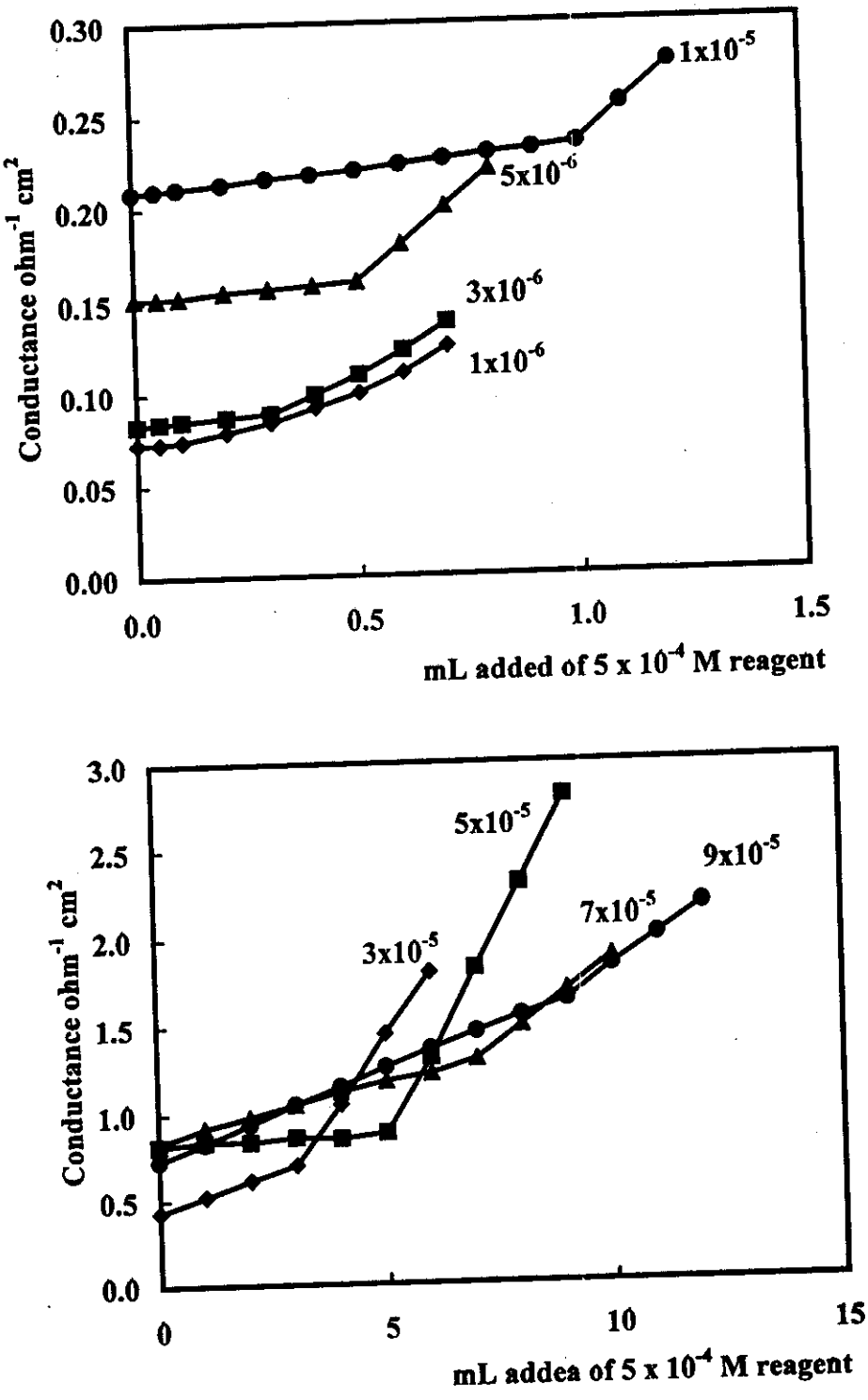


Fig. (19): Conductimetric titration of different concentration of Neom.SO_4using 5 x 10^{-4} M $(NH_4)_2[Ni(SCN)_4]$

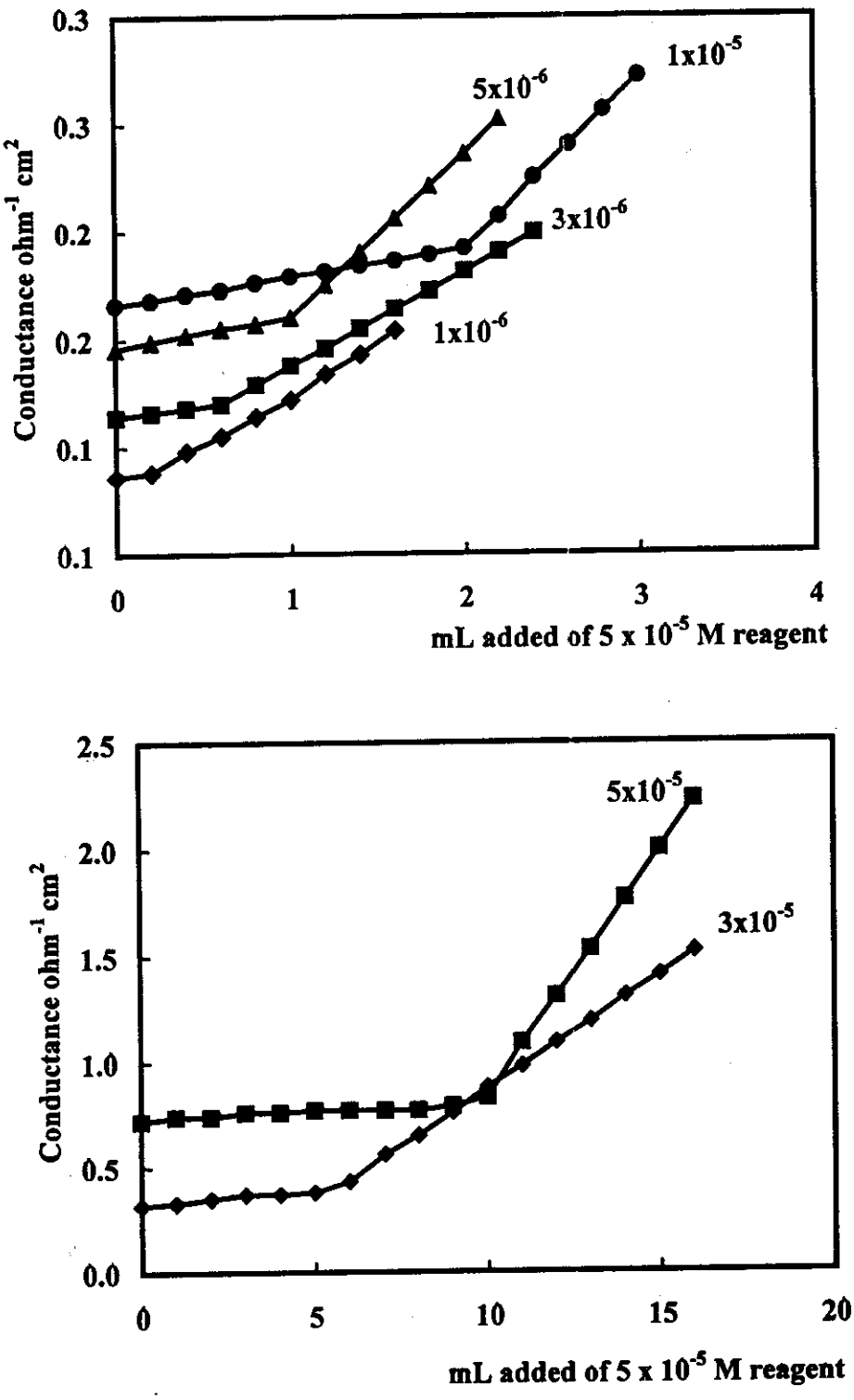
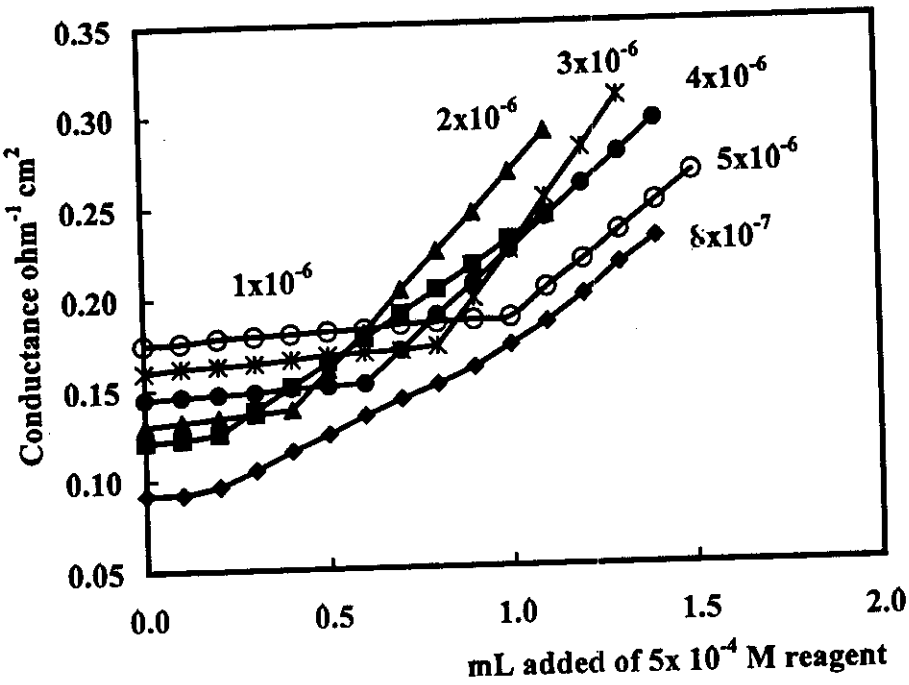


Fig. (20): Conductimetric titration of different concentration of Tobr.SO_4 using 5 x 10^{-5} M $(NH_4)_2[Ni(SCN)_4]$



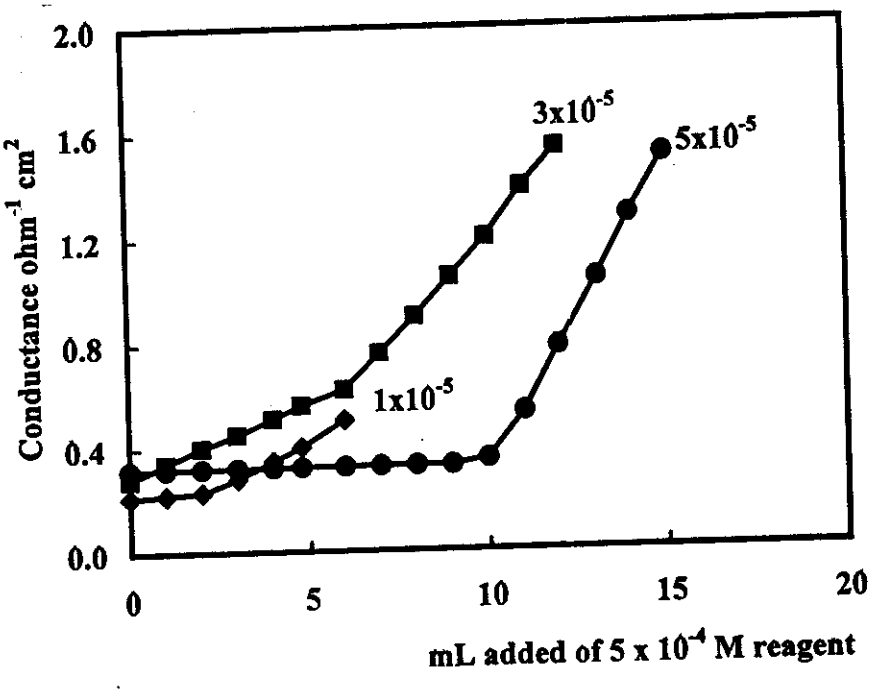
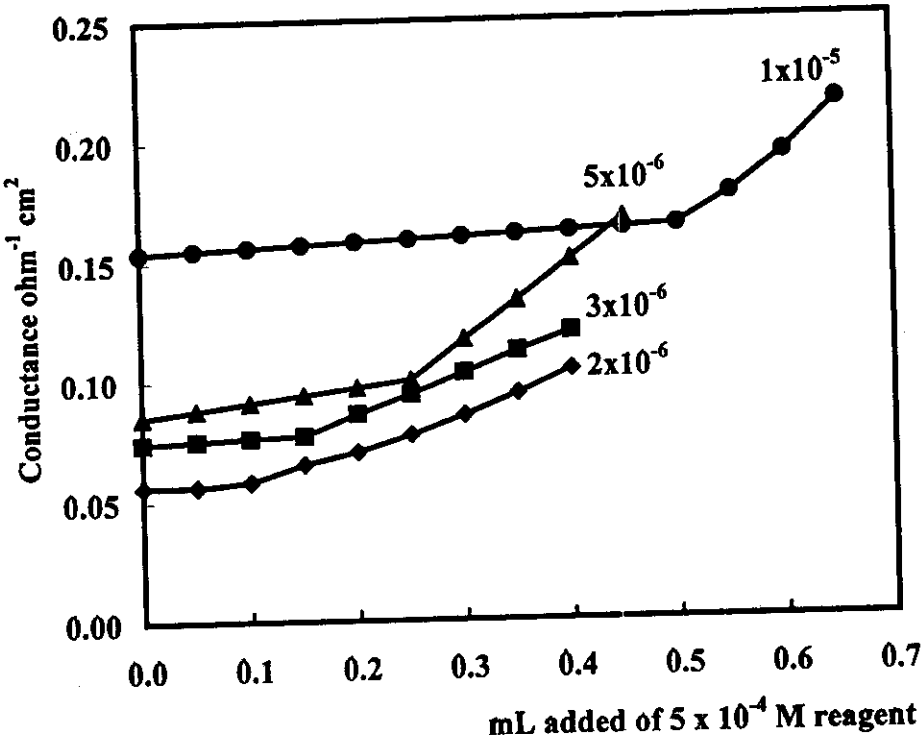


Fig. (21): Conductimetric titration of different concentration of Vanc.Cl using 5 x 10^{-4} M $(NH_4)_2[Ni(SCN)_4]$



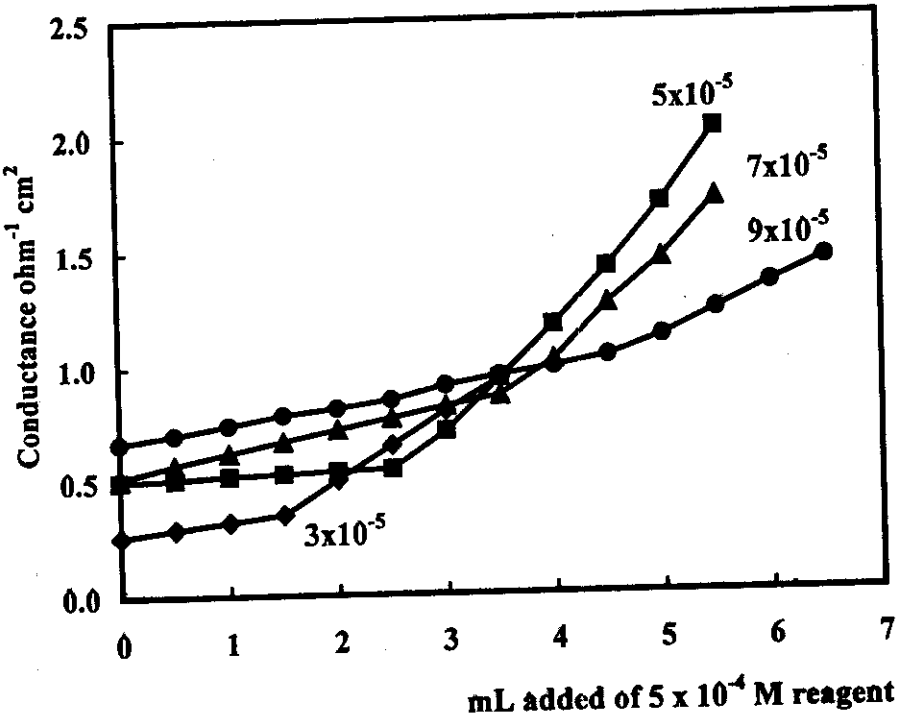
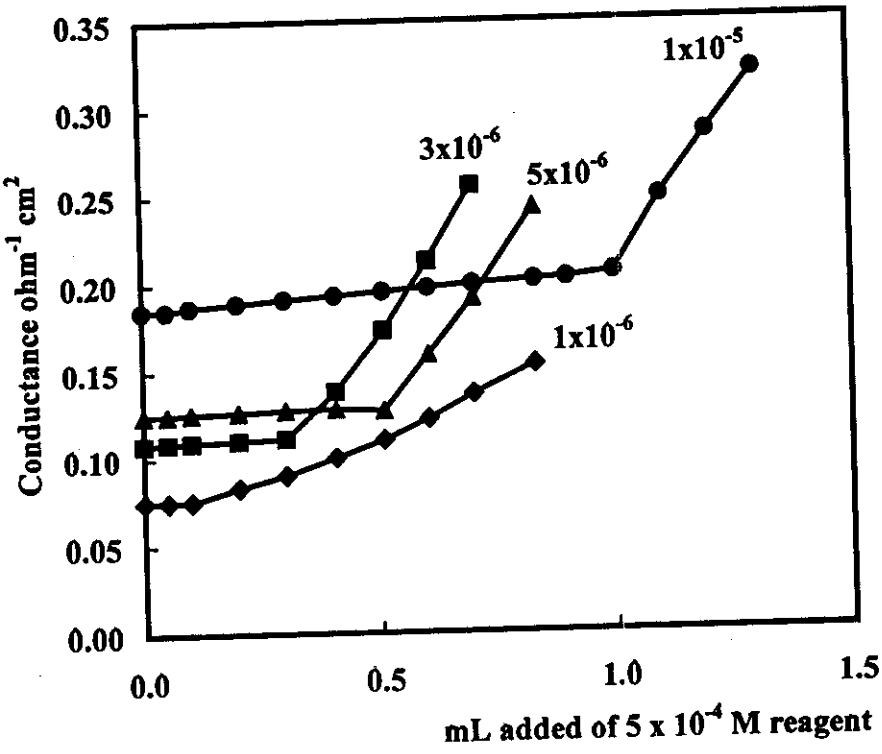


Fig. (22): Conductimetric titration of different concentration of Amik.SO_4using 5 x 10^{-4} M $Na_2[Fe(CN)_5NO]$



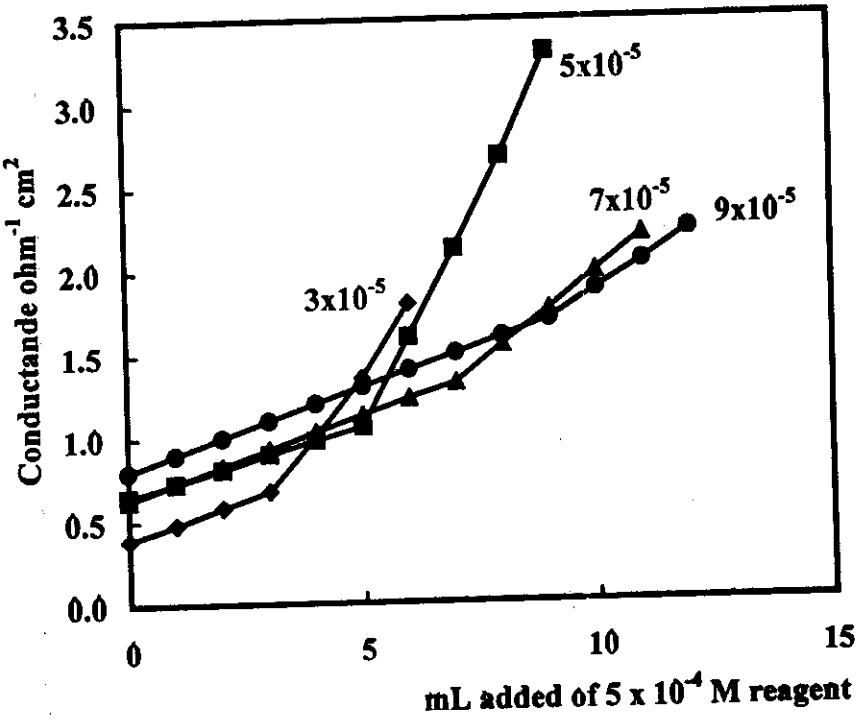
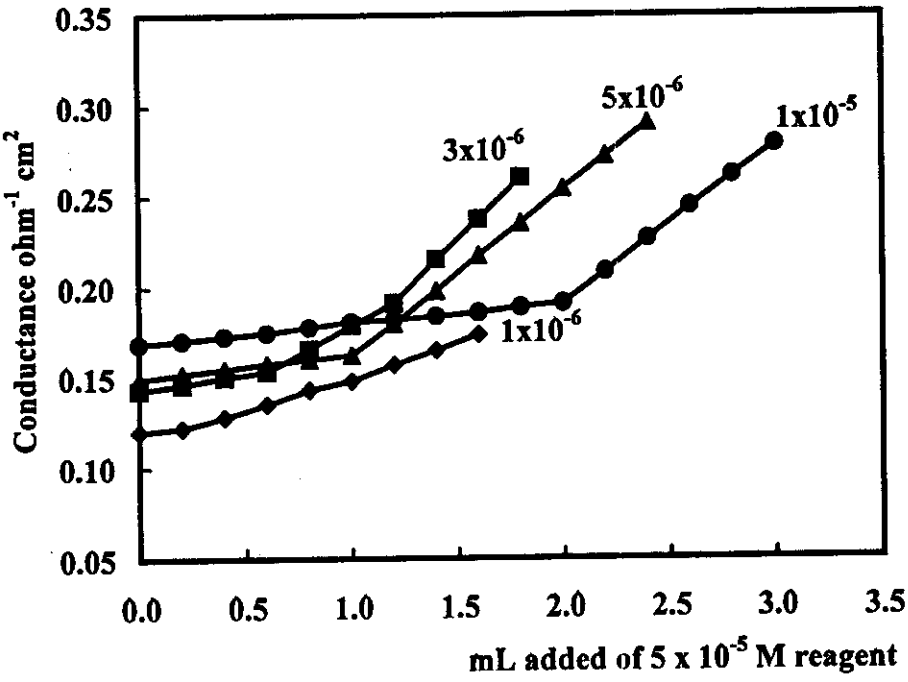


Fig. (23): Conductimetric titration of different concentration of Neom.SO_4 using 5 x 10^{-4} M $Na_2[Fe(CN)_5NO]$



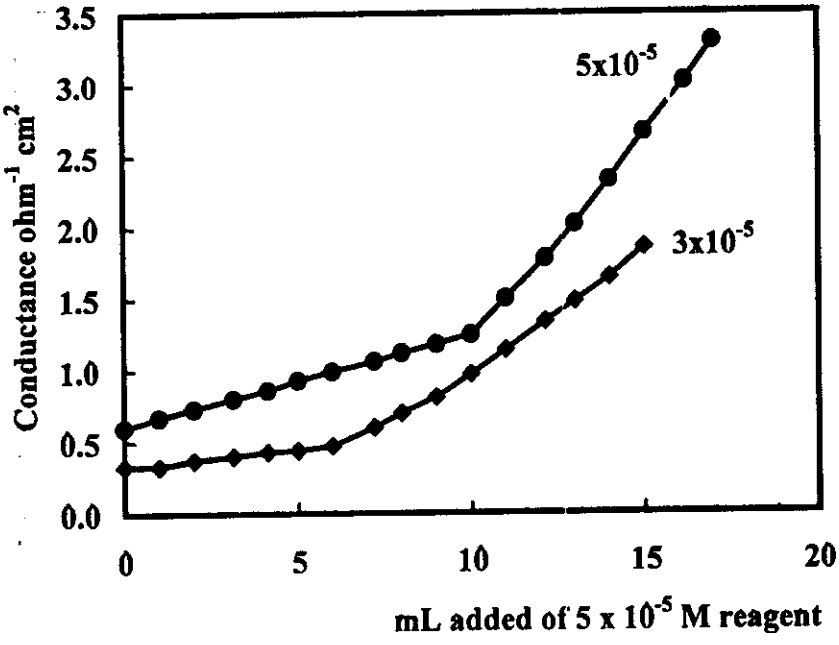
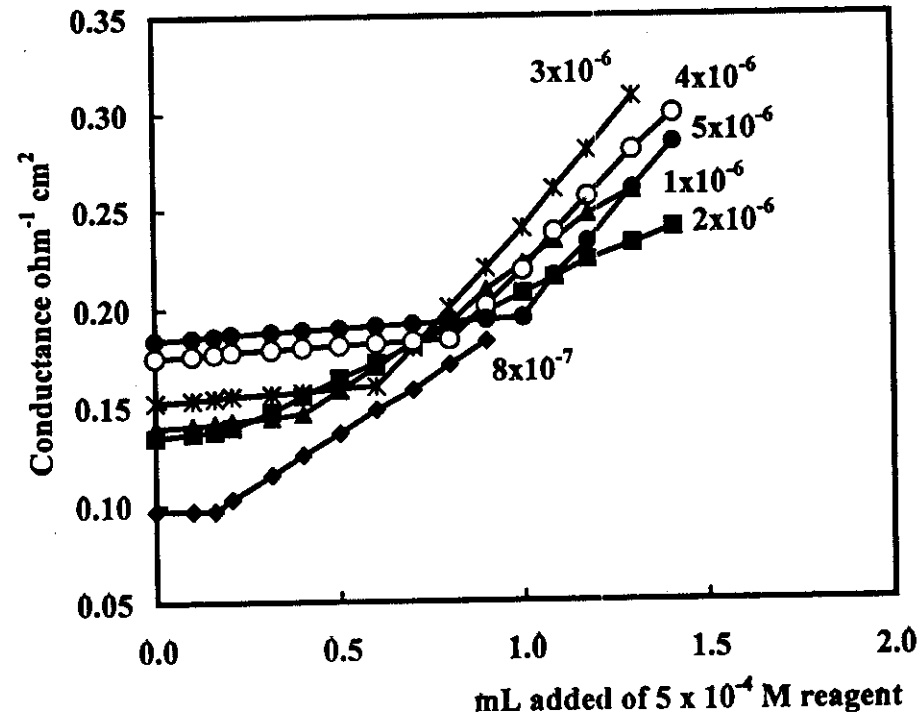


Fig. (24): Conductimetric titration of different concentration of Tobr.SO_4 using 5 x 10^{-5} M $Na_2[Fe(CN)_5NO]$



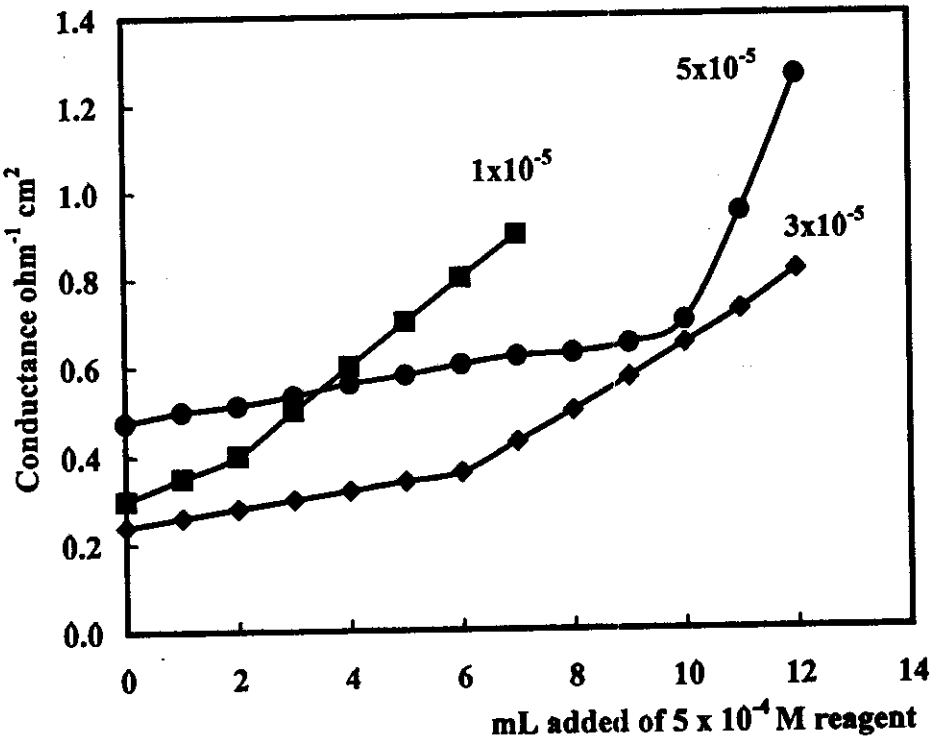


Fig. (25): Conductimetric titration of different concentration of Vánc.Cl using 5 x 10^{-4} M $Na_2[Fe(CN)_5NO]$

and 99.25-101.07%, respectively. As a result, the methods are applied successfully in the above mentioned ranges.

3.1.5. Conductimetric determination of the investigated drugs in pharmaceutical preparations and in urine

The proposed methods were applied to the determination of Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.CL in pharmaceutical preparations (tablets, eye drops vials and lotion). The results in [Table (4)] were compared with those obtained from the official methods [64,65] based on microbiological techniques. This comparison indicated that the proposed and official methods are of equal accuracy. The proposed methods are simple, time saving (15-30 min compared with at least 1.0 hr for the official methods) and sensitive, thereby encouraging their application in quality control of these aminoglycoside antibiotics in tablets, eye drops, vials and lotion preparations. In case of urine there was no interference using the cited reagents.

Table (2): Conductimetric determination of Amik.SO_4 and Neom.SO_4 in pure forms

Reagent	Amik.SO_4				Neom.SO_4			
	Taken $\mu g\ mL^{-1}$	Found[a] $\mu g\ mL^{-1}$	Recovery %	S.D. %	Taken $\mu g\ mL^{-1}$	Found[a] $\mu g\ mL^{-1}$	Recovery %	S.D. %
Ammonium	8.0	8.01	100.13	0.04	10.0	9.94	99.40	0.07
reineckate	16.0	15.95	99.69	0.06	20.0	19.88	99.40	0.04
	24.0	23.90	99.58	0.08	30.0	30.20	100.67	0.09
	30.0	30.25	100.83	0.05	40.0	39.75	99.38	0.05
	36.0	36.20	100.65	0.07	50.0	50.30	100.60	0.08
	42.0	41.75	99.40	0.09	60.0	60.40	100.67	0.06
Potassium	12.0	12.10	100.83	0.05	11.0	11.10	100.91	0.08
ferrocyanide	24.0	23.85	99.38	0.03	22.0	21.85	99.32	0.03
	36.0	36.25	100.69	0.09	33.0	32.80	99.39	0.07
	48.0	48.30	100.63	0.08	44.0	44.25	100.57	0.09
	60.0	59.70	99.50	0.06	55.0	55.40	100.73	0.07
	70.0	69.70	99.43	0.04	66.0	65.65	99.47	0.05
Cobalt	4.0	3.98	99.50	0.04	9.0	8.95	99.44	0.03
thiocyanate	8.0	7.95	99.38	0.03	18.0	17.88	99.33	0.05
	12.0	12.10	100.83	0.08	27.0	27.10	100.37	0.07
	16.0	16.15	100.94	0.06	36.0	36.20	100.55	0.06
	20.0	19.85	99.25	0.09	45.0	44.80	99.56	0.04
	24.0	24.15	100.62	0.07	54.0	54.40	100.74	0.08
Nickel	5.0	5.03	100.60	0.05	7.0	6.95	99.29	0.04
thiocyanate	10.0	9.98	99.80	0.03	14.0	14.15	101.07	0.08
	15.0	14.90	99.33	0.07	21.0	21.20	100.95	0.07
	20.0	20.15	100.75	0.09	28.0	28.30	100.07	0.06
	25.0	24.85	99.40	0.06	35.0	34.75	99.29	0.09
	30.0	30.25	100.83	0.05	42.0	41.65	99.17	0.04
Sodium	10.0	9.99	99.90	0.03	13.0	13.10	100.77	0.09
nitroprusside	20.0	20.15	100.75	0.06	26.0	26.25	100.96	0.07
	30.0	29.85	99.50	0.05	39.0	39.30	100.77	0.05
	40.0	39.80	99.50	0.08	52.0	51.75	99.52	0.03
	50.0	49.75	99.50	0.04	65.0	64.70	99.54	0.04
	60.0	60.30	100.50	0.07	78.0	78.50	100.64	0.06

[a]: Average of six determinations

Table (3): Conductimetric determination of Tobr.SO_4 and Vanc.Cl in pure forms

Reagent	Tobr.SO_4				Vanc.Cl			
	Taken $\mu g\ mL^{-1}$	Found[a] $\mu g\ mL^{-1}$	Recovery %	S.D. %	Taken $\mu g\ mL^{-1}$	Found[a] $\mu g\ mL^{-1}$	Recovery %	S.D. %
Ammonium reineckate	5.0	5.04	100.80	0.05	7.0	6.96	99.42	0.04
	10.0	9.92	99.20	0.07	14.0	14.10	100.71	0.07
	15.0	14.90	99.33	0.04	21.0	20.88	99.43	0.05
	20.0	19.85	99.25	0.06	28.0	28.20	100.70	0.09
	25.0	25.30	101.20	0.03	35.0	34.75	99.29	0.08
	30.0	30.30	101.00	0.08	42.0	42.30	100.71	0.06
Potassium ferrocyanide	13.0	13.20	101.54	0.04	9.0	9.06	100.67	0.07
	26.0	26.30	101.15	0.06	18.0	17.90	99.44	0.05
	39.0	39.50	101.28	0.03	27.0	27.20	100.74	0.03
	52.0	51.60	99.23	0.07	36.0	35.75	99.31	0.06
	65.0	64.50	99.23	0.09	45.0	45.35	100.78	0.08
	78.0	77.30	99.10	0.06	54.0	53.65	99.35	0.03
Cobalt thiocyanate	6.0	6.05	100.83	0.05	11.0	10.92	99.27	0.04
	12.0	11.94	99.50	0.08	22.0	21.85	99.32	0.07
	18.0	18.10	100.55	0.04	33.0	33.25	100.76	0.09
	24.0	24.25	101.04	0.07	44.0	43.70	99.32	0.05
	30.0	29.50	98.33	0.05	55.0	55.40	100.73	0.08
	36.0	35.50	98.61	0.03	66.0	65.65	99.47	0.04
Nickel thiocyanate	9.0	8.95	99.44	0.04	12.0	11.90	99.17	0.07
	18.0	18.20	101.11	0.06	24.0	23.85	99.38	0.05
	27.0	26.75	99.07	0.08	36.0	36.20	100.56	0.09
	36.0	36.30	100.83	0.09	48.0	48.30	100.63	0.08
	45.0	44.64	99.20	0.05	60.0	60.51	100.25	0.06
	54.0	54.50	100.92	0.04	72.0	71.70	99.58	0.03
Sodium nitroprusside	7.0	6.95	99.29	0.03	12.0	12.10	100.83	0.03
	14.0	14.12	100.86	0.08	24.0	23.85	99.38	0.07
	21.0	21.20	100.95	0.06	36.0	36.25	100.69	0.08
	28.0	27.75	99.11	0.07	48.0	48.30	100.63	0.09
	35.0	34.60	98.85	0.09	60.0	60.35	100.58	0.06
	42.0	41.50	98.81	0.05	72.0	71.70	99.58	0.04

[a]: Average of six determinations

3.2. Composition and structure of the ion-pairs

The ion-pairs are prepared as previously described under the experimental part. The resulting ion-pairs were subjected to elemental analysis for C, H, N and metal content.

3.2.1. Elemental analysis of ion-pairs

The metal content of the ion-pairs was determined by AAS after their digestion in concentrated nitric acid [60]. A calibration curve prepared from a standard solution of each metal ion was used for this purpose.

The results of elemental analysis (C, H, N, and metal content), [Table (5)], are in good agreement with those required by the suggested formulae. These analyses have been made at the Microanalytical Center of Cairo University.

3.2.2. Factors influencing solubility of the ion-pair

3.2.2.1. Effect of pH on the solubility of ion-pair

The choice of a suitable pH value at which the ion-pairs exhibit the lowest solubility is of prime importance in the use of such ion-pairs in quantitative analysis. The effect of pH value on the solubility of the ion-pairs under investigation has been investigated using 0.1 M HCl or NaOH solution at different pH values (1.0 – 10). The solid ion-pairs were added to 25 mL of these solutions till saturation. The solution were shaken for 4-6 hrs and left to stand for one day for attaining a stable equilibrium. The solubility and solubility product of the ion-pairs are calculated from the metal

Table (4): Determination of Amik.SO_4, Neom.SO_4, Tobr.SO_4 in pharmaceutical preparations.

Dosage forms	Company	Label mg content	Fund [a]					Official
			Ammonium reineckate	Potassium ferrocyanide	Cobalt thiocyanate	Nickel thiocyanate	Sodium nitroprusside	
Likacin vial	EIPICO	500	494 t = 1.72 F = 3.37	504 t = 1.90 F = 4.05	502 t = 1.96 F = 4.08	506 t = 1.66 F = 3.20	498 t = 1.93 F = 4.08	510
Amikin vial	Squibb	500	505 t = 1.84 F = 3.65	498 t = 1.54 F = 3.00	495 t = 2.12 F = 4.50	503 t = 1.75 F = 3.40	504 t = 1.77 F = 3.40	488
	Squibb	100	99.2 t = 1.67 F = 3.25	99.4 t = 1.77 F = 3.49	99.3 t = 2.08 F = 4.22	100.8 t = 2.00 F = 4.12	100.4 t = 2.04 F = 4.25	98.5
Neo-Cortef eye drop	EIPICO	5.0/ mL	4.98 t = 1.90 F = 4.04	5.05 t = 2.08 F = 4.44	4.96 t = 1.93 F = 4.01	4.97 t = 1.53 F = 2.97	5.04 t = 1.69 F = 3.14	4.80
Neo-Medrol lotion	EIPICO	2.5 /mL	2.51 t = 1.81 F = 3.92	2.48 t = 1.80 F = 3.76	2.53 t = 1.85 F = 3.90	2.49 t = 1.44 F = 2.81	2.50 t = 1.72 F = 3.47	2.55
Neomycin tablets	Memphis	500	505 t = 1.58 F = 3.09	494 t = 1.93 F = 4.00	502 t = 1.64 F = 3.86	503 t = 1.63 F = 3.20	497 t = 1.85 F = 3.80	487
Tobcin vial	Memphis	20	20.04 t = 1.84 F = 3.65	20.06 t = 1.75 F = 3.20	19.80 t = 1.93 F = 4.08	19.79 t = 1.44 F = 2.81	19.95 t = 1.66 F = 3.20	20.02
Tobrin vial	EIPICO	20	20.01 t = 1.98 F = 4.05	19.85 t = 1.55 F = 2.98	19.95 t = 1.83 F = 3.77	20.05 t = 1.66 F = 3.88	19.98 t = 1.95 F = 4.01	19.98
Tobrin eye drop	EIPICO	5/mL	4.97 t = 1.53 F = 2.14	5.04 t = 1.69 F = 3.14	5.05 t = 1.08 F = 4.44	4.98 t = 1.90 F = 4.04	4.96 t = 1.93 F = 4.01	4.85

[a]: Average of six determination.

Table (5): Elemental analysis and Composition of the ion-pairs

Ion-pair	Formula	Molar Ratio	Colour	Elemental analysis (calculated)			
				%C	%H	%N	Metal
Neom-ferrocyanide	$[C_{23}H_{46}N_6O_{13}]_2[Fe(CN)_6]$	2:1	Blue	(43.33)	(6.38)	(17.49)	(3.87)
				43.60	6.45	17.45	4.00
Neom-cobaltthiocyanate	$[C_{23}H_{46}N_6O_{13}]_2[Co(SCN)_4]$	1:1	Brown	(35.79)	(5.07)	(15.45)	(6.50)
				35.90	5.10	15.40	6.60
Tobr-cobaltthiocyanate	$[(C_{18}H_{37}N_5O_9)_2][Co(SCN)_4]_5$	1:5	Pink	(38.17)	(3.09)	(17.57)	(12.32)
				38.20	3.15	17.50	12.30
Vanc-reineckate	$[C_{66}H_{75}Cl_2N_9O_{24}][Cr(NH_3)_2(SCN)_4]$	1:1	Brown	(47.58)	(4.58)	(11.88)	(2.94)
				47.50	4.65	11.80	2.90
Vanc-ferrocyanide	$[C_{66}H_{75}Cl_2N_9O_{24}]_4[Fe(CN)_6]$	4:1	Bright	(53.98)	(4.99)	(9.79)	(0.93)
			Blue	54.00	5.00	9.70	1.00
Vanc- cobaltthiocyanate	$[C_{66}H_{75}Cl_2N_9O_{24}]_2[Co(SCN)_4]$	2:1	Gray	(51.22)	(4.70)	(9.66)	(1.84)
				51.30	4.80	9.50	1.80
Vanc-nickelthiocyanate	$[C_{66}H_{75}Cl_2N_9O_{24}]_2[Ni(SCN)_4]$	2:1	yellow	(51.23)	(4.70)	(9.66)	(1.84)
				51.40	4.75	9.60	1.88
Vanc-nitroprusside	$[C_{66}H_{75}Cl_2N_9O_{24}]_2[Fe(CN)_5NO]$	2:1	Bale	(52.85)	(4.81)	(10.79)	(1.79)
			Green	53.00	4.85	10.70	1.85

concentration. The values of the solubilities and solubility products of ion-pairs at the optimum pH are collected in [Table (6)].

3.2.2.2. Solubility

Chemical compounds have different solubility depending on their nature, and at a given temperature only a limited amount of them can be solved in a given solvent. The solid phase and the solution saturated with the solute are in dynamic equilibrium, which means that, the amount solved per unit time is equal to the amount precipitated in this time. The equilibrium can be characterized by the concentration of the saturated solution. Clearly, a precipitate for gravimetric work must have a sufficiently low solubility so that the amount lost dose not seriously affect the outcome of the analysis where the quantity of substance being determined is low and where the demands for accuracy are high solubility losses may be of real concern.

The present study involves the determination of the optimum experimental conditions for quantitative precipitation of the ion-pairs formed from the reaction of the drugs under investigation (Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl) with different inorganic complexes. The method of determination is based on precipitating the drug inorganic complex ion-pair and measuring the equilibrium metal ion concentration present after precipitation. Accordingly, the quantitative determination of the cited drugs can be performed.

In this study, the amount of metal ion present in the filtrate is determined using AAS. The solubility of the ion-pair and the solubility product are then calculated.

3.2.2.3. Solubility product

When a compound is refereed to as insoluble, it is not completely insoluble but it is sparingly soluble. In general, analytical precipitates are salts, or other ionic compounds with low solubility, which dissociate into ions in their solutions. Generally, for the equilibrium

$$A_x B_y \longrightarrow xA^{y+} + yB^{x-}$$

The solubility product is given by the relation:

$$K_{SP} = [CA^x]^{y+} . [CB^y]^{x-}$$

This constant is generally applicable at the equilibrium conditions for saturated solutions of slightly soluble electrolytes and is taken as a measure for solubility of the compound from the knowledge of the value of the solubility product at a specific temperature, the solubility of the compound can be calculated.

The following investigations are concerned with different factors affecting the solubility of the ion-pairs formed between some pharmaceutical compounds and some inorganic complex ions. This aims essentially to use such ion-pairs in analysis of these pharmaceutical compounds.

Table (6): Solubility and solubility product values of ion-pair at the optimum pH values

Ion-pair	pH_i	pH_f	pS	pK_{SP}
$[Neom]_2[Fe(CN)_6]$	9.00	7.11	5.48	15.87
$[Neom]_2[Co(SCN)_4]$	10.00	9.50	5.36	10.72
$[Tobr][Co(SCN)_4]_5$	5.00	3.50	5.45	27.55
$[Vanc][Cr(NH_3)(SCN)_4]$	4.00	3.13	5.06	10.12
$[Vanc]_4[Fe(CN)_6]$	5.00	3.40	5.81	26.66
$[Vanc]_2[Co(SCN)_4]$	9.00	7.75	5.83	16.89
$[Vanc]_2[Ni(SCN)_4]$	9.00	7.75	5.68	16.45
$[Vanc]_2[Fe(CN)_5NO]$	10.00	8.00	5.48	15.86

pH_i: Initial pH value

pH_f: pH value at equilibrium

pS: - log solubility

pK_{SP}: - log solubility product

3.3. Atomic absorption spectrometric studies

Analytical chemistry is a very important branch, especially in drug analysis where without quality control and quality assurance, the dosage forms cannot be used. Thus the analyst needs rapid, easy, inexpensive, sensitive, selective and accurate method of analysis. These properties could be achieved by the use of ion-pair complexes in quantitative analysis of pharmaceutical compounds.

The drugs under investigation are determined quantitatively by the indirect measurements of the equilibrium metal ion concentration

present after precipitation of the drugs in the form of insoluble ion-pairs using atomic absorption spectrometry.

The proposed methods depends on the addition of ammonium reineckate, potassium ferrocyanide, cobalt thiocyanate, nickel thiocyanate and sodium nitroprusside solution to sulphate or hydrochloride solutions of the drug to form the ion-pairs, under the most favourable conditions for the formation of the ion-pair. The excess soluble metal complex is then determined in the filtrate using the AAS.

3.3.1. Determination of the studied drugs in pure solutions using AAS

Determination of Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl in pure solutions is performed within the range 1.1726-11.5896 mg/25 mL using ammonium reineckate and 1.1726-14.1249 mg/25 mL using potassium ferrocyanide as ion pair forming reagent. The results given in [Tables (7-10)] showed that, the present method is highly precise and accurate. The relative standard deviations and recoveries percent are within the ranges 0.94 to 1.78% and 99.36-101.24% using reineckate and 0.96 to 1.83% and 98.22 to 100.74% using ferrocyanide. Also, it can be applied for very low concentration of the drugs as shown by their detection limits.

For the quantitative analysis of Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl using cobalt thiocyanate, nickel thiocyanate or sodium nitroprusside as ion-pair forming reagent, the working

ranges were found to be 0.4544-12.1159 mg/25 mL. The results given showed that, the present method is highly precise and accurate as indicated from relative standard deviation and recovery values ranging from 0.89-1.87% and 98.46-101.75%. Also, the method can be applied for very low concentration of the drugs as recorded by their detection limits [Tables (7-10)].

3.3.2. Determination of the investigated drugs in dosage forms and urine samples

The determination of Amik.SO_4, Neom.SO_4, Tobr.SO_4, and Vanc.Cl in dosage forms (tablets, eye drops, vials and lotion) and in urine was performed the relative standard deviation and recovery percentage were calculated and recorded in [Table (11)]. These results indicated that, the present methods are accurate and precise. However, the suggested methods are in good agreement with the official methods (base on microbiological assay). The present methods are applied within a wider range than the method described by Amin[14], Confino[30], Gupta[31] and Sampath[45].

From all the above results, it is shown that, the present methods are highly accurate and precise for determination of a wide concentration range of the cited drugs in pure forms, dosage forms and urine samples.

Table (7): Determination of the concentration of Amik.SO_4 in the pure forms, its characteristic concentrations and detection limits using AAS.

mg/ 25 mL		Recovery	RE	RSD	Charact. Conc.	Detection limit
Taken	Found *	%	%	%	µg mL^{-1}	µg mL^{-1}
$NH_4[Cr(NH_3)_2(SCN_4)]$						
1.1726	1.173	100.03	-0.37	1.58	23.20	15.60
2.3452	2.361	100.067	-0.29	1.51		
3.5178	3.514	99.89	-0.61	1.63		
4.6904	4.715	100.52	-0.47	1.56		
5.8630	5.859	99.93	-0.35	1.71		
$K_4[Fe(CN)_6]$						
1.5635	1.561	99.84	-0.45	1.39	31.10	20.75
3.1269	3.122	99.84	-0.53	1.61		
4.6904	4.725	100.74	-0.39	1.52		
6.2539	6.258	100.07	-0.40	1.48		
7.8174	7.805	99.84	-0.61	1.72		
$(NH_4)_2[CO(SCN)_4]$						
0.7817	0.782	100.04	-0.29	1.46	15.30	10.20
1.9543	1.951	99.83	0.36	1.59		
3.1269	3.134	100.23	-0.48	1.72		
4.2995	4.305	100.13	-0.59	1.80		
5.4721	5.481	100.16	-0.62	1.55		
$(NH_4)_2[Ni(SCN)_4]$						
0.9772	0.979	99.26	-0.27	1.33	32.80	26.00
1.9543	1.958	100.19	-0.36	1.47		
2.9315	2.927	99.85	-0.58	1.66		
4.8858	4.893	100.15	-0.43	1.50		
$Na_2[Fe(CN)_5NO]$						
0.5471	0.546	99.79	-0.31	1.60	10.65	7.10
1.3678	1.375	100.52	-0.46	1.43		
2.7355	2.739	100.12	-0.53	1.59		
4.1033	4.111	100.19	-0.46	1.71		
5.4711	5.505	100.62	-0.39	1.58		

* Average of six determinations

RE: relative error

RSD: relative standard deviation

Table (8): Determination of the concentration of Neom.SO_4 in the pure forms, its characteristic concentrations and detection limits using AAS.

mg/ 25 mL		Recovery	RE	RSD	Charact. Conc.	Detection limit
Taken	Found*	%	%	%	µg mL^{-1}	µg mL^{-1}
$NH_4[Cr(NH_3)_2(SCN_4)]$						
1.3633	1.375	100.85	-0.21	0.94	54.00	36.10
2.7266	2.712	99.46	-0.34	1.05		
4.0899	4.068	99.46	-0.41	1.63		
5.4532	5.492	100.71	-0.28	1.08		
6.8165	6.777	99.42	-0.56	1.72		
$K_4[Fe(CN)_6]$						
0.9089	0.915	100.70	-0.36	1.48	18.00	11.90
2.7266	2.744	100.64	-0.43	1.56		
4.5453	4.508	99.20	-0.27	1.11		
6.3606	6.326	99.46	-0.40	1.38		
8.1798	8.212	100.40	-0.25	1.06		
$(NH_4)_2[CO(SCN)_4]$						
0.5471	0.550	100.53	-0.48	1.76	11.00	7.25
1.6413	1.616	98.46	-0.32	1.44		
3.2826	3.323	101.23	-0.39	1.61		
4.9239	5.000	101.55	-0.26	1.22		
6.6552	6.610	99.32	0.20	0.89		
$(NH_4)_2[Ni(SCN)_4]$						
0.5544	0.449	98.81	-0.35	1.42	9.00	5.95
2.2722	2.221	100.39	-0.53	1.79		
4.5443	4.563	100.41	-0.28	1.13		
6.8165	6.800	99.76	-0.19	0.85		
9.0887	9.131	100.47	-0.38	1.50		
$Na_2[Fe(CN)_5NO]$						
0.9089	0.915	100.67	-0.24	1.00	17.95	11.85
2.2722	2.256	99.29	-0.30	1.45		
3.6356	3.600	99.02	-0.48	1.68		
4.9990	5.055	101.12	-0.36	1.27		
6.3623	6.328	99.46	-0.51	1.66		

* Average of six determinations

RE: relative error

RSD: relative standard deviation

Table (9): Determination of the concentration of Tobr.SO_4 in the pure forms, its characteristic concentrations and detection limits using AAS

mg/ 25 mL		Recovery	RE	RSD	Charact. Conc.	Detection limit
Taken	Found*	%	%	%	µg mL^{-1}	µg mL^{-1}
$NH_4[Cr(NH_3)_2(SCN_4)]$						
2.1381	2.144	100.28	-0.41	1.35	42.20	28.40
4.2762	4.268	99.81	-0.56	1.53		
6.4143	6.400	99.78	-0.38	1.44		
8.5524	8.591	100.46	-0.56	1.78		
10.6905	10.656	99.68	-0.47	1.56		
$K_4[Fe(CN)_6]$						
1.4254	1.400	98.22	-0.29	0.96	28.60	19.00
3.5635	3.551	99.65	-0.53	1.59		
6.4143	6.444	100.46	-0.44	1.48		
9.9778	10.000	100.22	-0.38	1.59		
12.1159	12.085	99.74	-0.61	1.83		
$(NH_4)_2[CO(SCN)_4]$						
0.9977	0.988	99.03	-0.33	1.45	19.75	13.30
2.9931	3.000	100.23	-0.57	1.80		
4.9885	4.955	99.33	-0.46	1.51		
7.4828	7.506	100.31	-0.68	1.75		
8.9793	9.010	100.34	-0.28	1.17		
$(NH_4)_2$ $[Ni(SCN)_4]$						
1.1400	1.160	101.75	-0.39	1.48	22.50	15.10
2.8506	2.900	101.73	-0.55	1.69		
5.7011	5.700	99.98	-0.71	1.87		
8.5517	8.515	99.57	-0.44	1.49		
10.2621	10.222	99.56	-0.34	1.40		
$Na_2[Fe(CN)_5NO]$						
1.4254	1.411	98.99	-0.47	1.35	28.50	19.05
3.4207	3.445	100.71	-0.63	1.48		
5.1310	5.160	100.57	-0.36	1.51		
7.9816	8.033	100.64	-0.55	1.70		
9.6919	9.600	99.05	-0.40	1.53		

* Average of six determinations

RE: relative error

RSD: relative standard deviation

Table (10): Determination of the concentration of Vanc.Cl in the pure forms, its characteristic concentrations and detection limits using AAS.

mg/ 25 mL		Recovery	RE	RSD	Charact. Conc.	Detection limit
Taken	Found*	%	%	%	$\mu g\ mL^{-1}$	$\mu g\ mL^{-1}$
$NH_4[Cr(NH_3)_2(SCN_4)]$						
2.1730	2.170	99.86	-0.43	1.27	42.95	29.00
4.3460	4.360	100.32	-0.56	1.48		
6.5190	6.600	101.24	-0.36	1.30		
8.6900	8.640	99.42	-0.61	1.73		
13.0380	12.955	99.36	-0.50	1.65		
$K_4[Fe(CN)_6]$						
1.4487	1.424	98.30	-0.29	1.03	29.00	19.25
3.6217	3.596	99.29	-0.53	1.68		
5.7947	5.834	100.68	-0.41	1.30		
9.4163	9.450	100.36	-0.63	1.80		
11.5893	11.518	99.38	-0.36	1.42		
$(NH_4)_2[CO(SCN)_4]$						
1.014	1.00	98.62	-0.38	1.45	20.15	13.50
2.535	2.500	98.62	-0.27	0.76		
4.056	4.100	101.08	-0.48	1.54		
6.084	6.155	101.17	-0.69	1.77		
9.126	9.000	99.62	-0.83	1.66		
$(NH_4)_2[Ni(SCN)_4]$						
1.1589	1.145	99.66	-0.41	1.29	23.00	15.40
2.8971	2.857	98.62	-0.55	1.48		
5.2149	5.253	100.73	-0.34	1.62		
8.112	8.202	101.109	-0.60	1.74		
10.4297	10.400	99.72	-0.48	1.59		
$Na_2[Fe(CN)_5NO]$						
1.4487	1.463	100.99	-0.36	1.47	28.90	19.20
4.3461	4.306	99.08	-0.57	1.70		
7.9679	8.004	100.45	-0.44	1.53		
10.8653	10.918	100.49	-0.30	1.46		
11.590	11.500	99.22	-0.64	1.78		

* Average of six determinations

RE: relative error

RSD: relative standard deviation

Table (11): Determination of the investigated drugs in pharmaceutical dosage forms and urine using AAS

Reagent	Taken mg	Recovery %	RE %	RSD %
$NH_4[Cr(NH_3)_2(SCN)_4]$				
Likacin vial (500 mg)	15.00	99.33	-0.99	0.073
Amikin vial (500 mg)	20.00	68.80	-0.05	0.056
Amikin vial (100 mg)	25.00	99.00	-1.05	0.044
Neo-Cortef (5 mg/mL)	5.00	98.50	-1.15	0.067
Neo-Medrol (2.5 mg/mL)	3.00	100.85	-0.94	0.058
Neomycin tablet (500 mg)	30.00	101.25	-1.17	0.039
Neomycin powder (125 mg)	15.00	98.75	-0.96	0.053
Tobracin vial (20 mg)	20.00	100.87	-1.24	0.081
Tobrin vial (20 mg)	15.00	98.50	-1.23	0.078
Tobrin eye drop (5mg/mL)	2.50	98.30	-0.84	0.050
Urine 1	3.00	99.75	-0.84	0.063
Urine 2	7.50	98.90	-0.88	0.075
$K_4[Fe(CN)_6]$				
Likacin vial (500 mg)	25.00	98.20	-1.00	0.058
Amikin vial (500 mg)	15.00	99.67	-1.00	0.081
Amikin vial (100 mg)	20.00	100.80	-1.17	0.038
Neo-Cortef (5 mg/mL)	10.00	101.20	-0.93	0.054
Neo-Medrol (2.5 mg/mL)	5.00	101.00	-1.01	0.065
Neomycin tablet (500 mg)	2.00	101.60	-0.86	0.047
Neomycin powder (125 mg)	10.00	99.30	-0.84	0.073
Tobracin vial (20 mg)	15.00	98.30	-0.94	0.069
Tobrin vial (20 mg)	10.00	98.00	-1.10	0.047
Tobrin eye drop (5 mg/mL)	5.00	99.50	-0.36	0.059
Urine 1	5.00	100.70	-0.90	0.066

Table (11): contd.

Urine 2	10.0	101.50	-0.31	0.049
$(NH_4)_2[Co(SCN)_4]$				
Likacin vial (500 mg)	10.00	98.50	-0.55	0.068
Amikin vial (500 mg)	20.00	100.80	-0.88	0.052
Amikin vial (100 mg)	10.00	99.10	-1.10	0.041
Neo-Cortef (5 mg/mL)	10.00	100.70	-0.94	0.036
Neo-Medrol (2.5 mg/mL)	5.00	100.80	-1.15	0.080
Neomycin tablet (500 mg)	10.00	98.90	-0.91	0.073
Neomycin powder (125 mg)	15.00	99.33	-1.09	0.049
Tobracin vial (20 mg)	20.00	99.80	-0.83	0.093
Tobrin vial (20 mg)	7.50	97.90	-0.86	0.078
Tobrin eye drop (5 mg/mL)	15.00	98.50	-1.01	0.071
Urine 1	9.00	98.95	-0.79	0.056
Urine 2	4.00	98.85	-0.87	0.033

* Average of six determinations

RE: relative error

3.4. Absorption spectra of the studied drugs using potassium ferrocyanide as inorganic reagents

In order to investigate the optimum reaction conditions for the reaction of the studied drugs with potassium ferrocyanide, as the effect of different experimental variables, the following studies should be taken into consideration. At the optimum conditions of pH, time, temperature, solvents and sequence of additions, the acid media oxidize the ferrous ions present in potassium ferrocyanide to ferric ion forming ferri ferrocyanide (Prussian blue) and under the action of the drug, it form an ion pair complex, which absorb

maximally at 523, 523, 504 and 507 nm for Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl, respectively.

$$K_4[Fe(CN)_6] \xrightarrow[\text{Heat}]{\text{Acid}} Fe_4[Fe(CN)_6]_3$$

The blue colour appeared was measured calorimetrically at λ_{max} 523, 523, 504 and 507 nm for Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl, respectively.

This behavior is applied for the determination of Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl through measuring the absorbance of the ion pair coloured product at the optimum wavelength. The following parameters affecting the reaction development were studied.

3.4.1 Effect of acid concentration

The effect of acid on the colour development between aminoglycoside drugs and potassium ferrocyanide was studied in different acid media (hydrochloric, sulphuric, phosphoric, acetic and perchloric acids). On using perchloric and acetic acids, the results were poorly reproducible and give low absorptivity on standing long time. The absorption spectra of the resulting solutions were recorded in the visible range at λ_{max} 523, 523, 504 and 507 nm for Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl, respectively, against a reagent blank solution of the same acid reagent concentration [Figs. (26-29)].

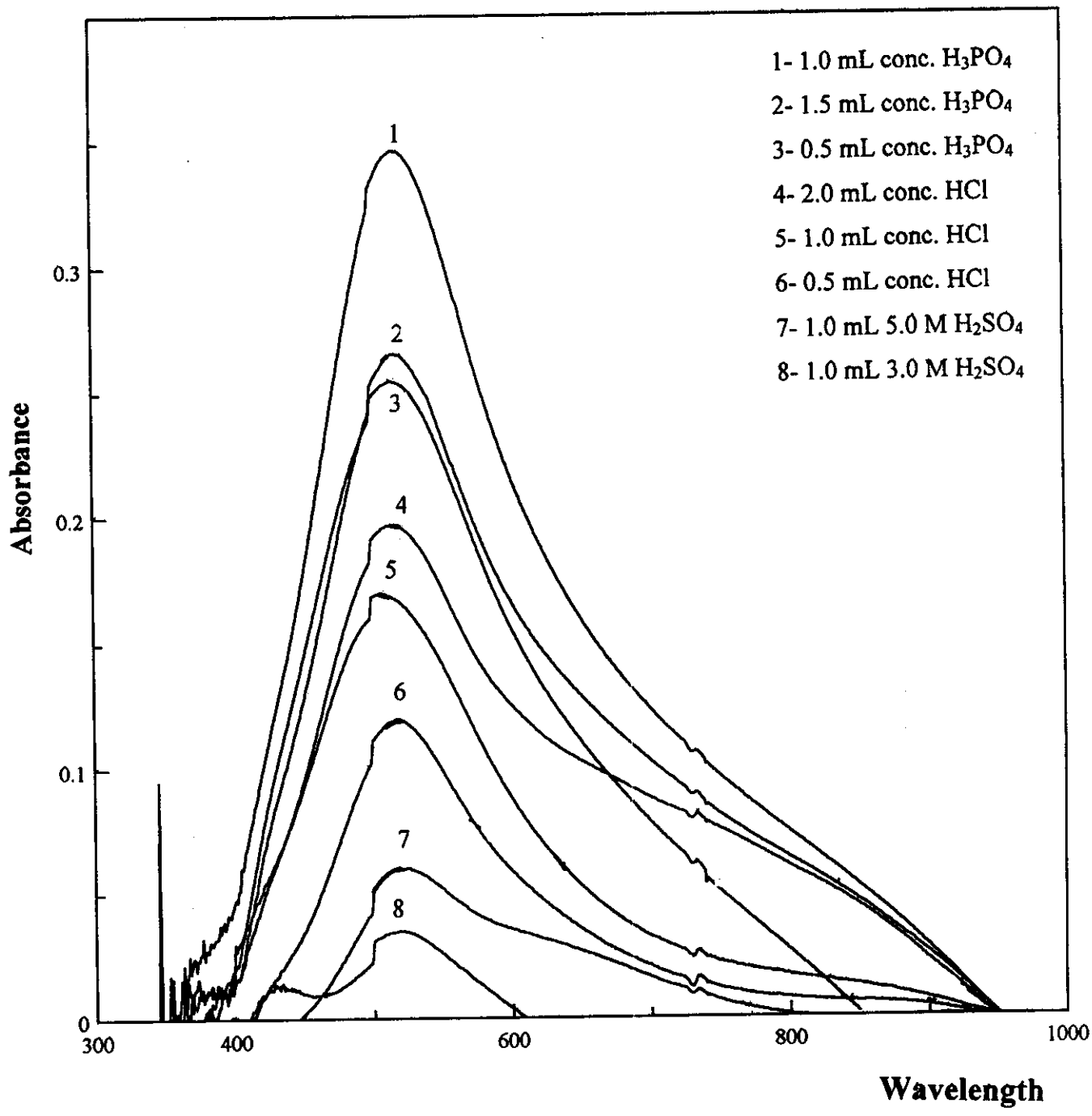


Fig. (26): Absorption spectra of 50 μg mL^{-1} of Amik.SO_4 using 5×10^{-2} M $K_4[Fe(CN)_6]$ at different acid media.

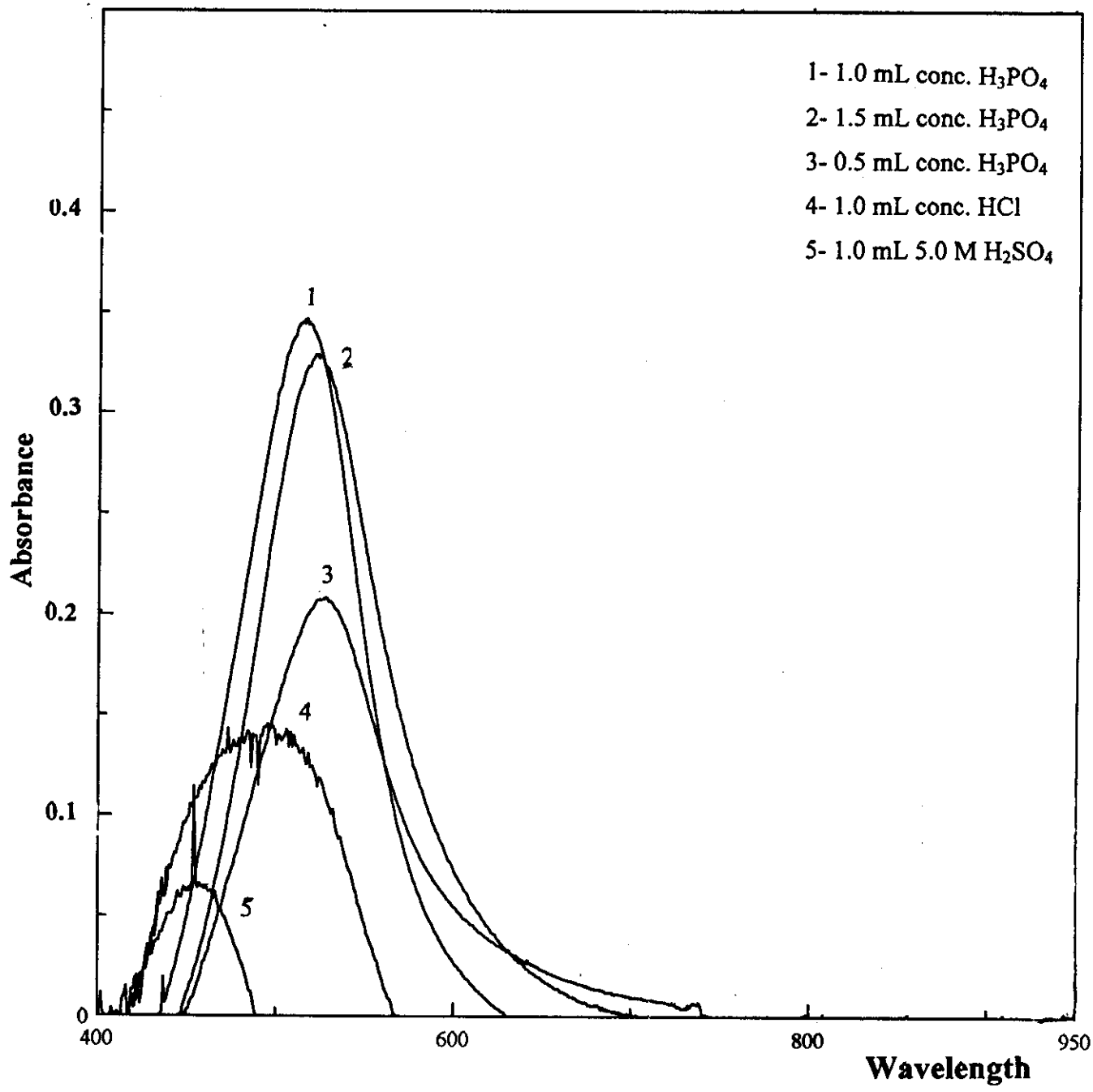


Fig. (27): Absorption spectra of 6.0 μg mL^{-1} of Neom.SO_4 using 5×10^{-2} M $K_4[Fe(CN)_6]$ at different acid media.

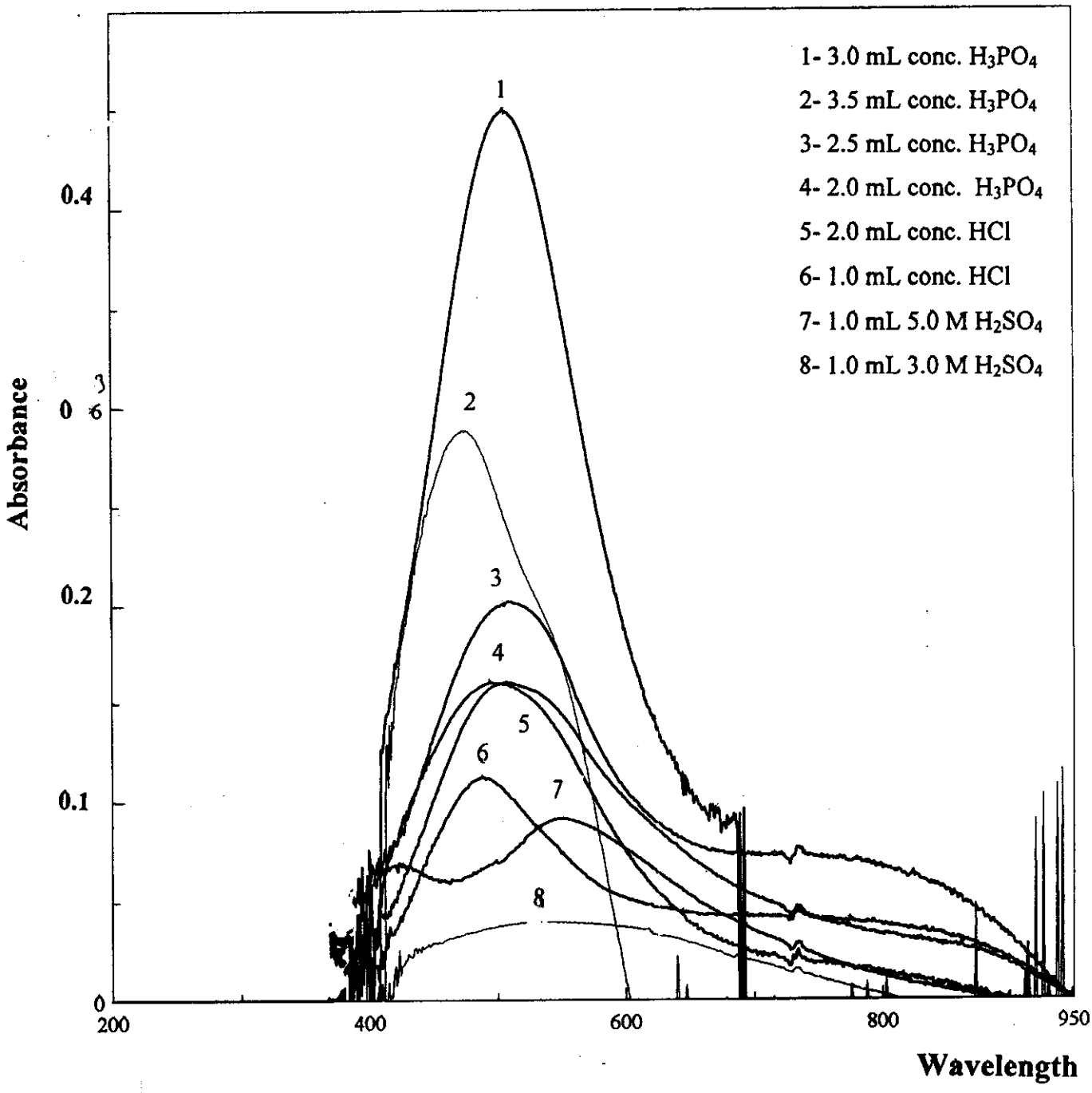


Fig. (28): Absorption spectra of 6.0 μg mL^{-1} of Tobr.SO_4 using 5×10^{-2} M $K_4[Fe(CN)_6]$ at different acid media.

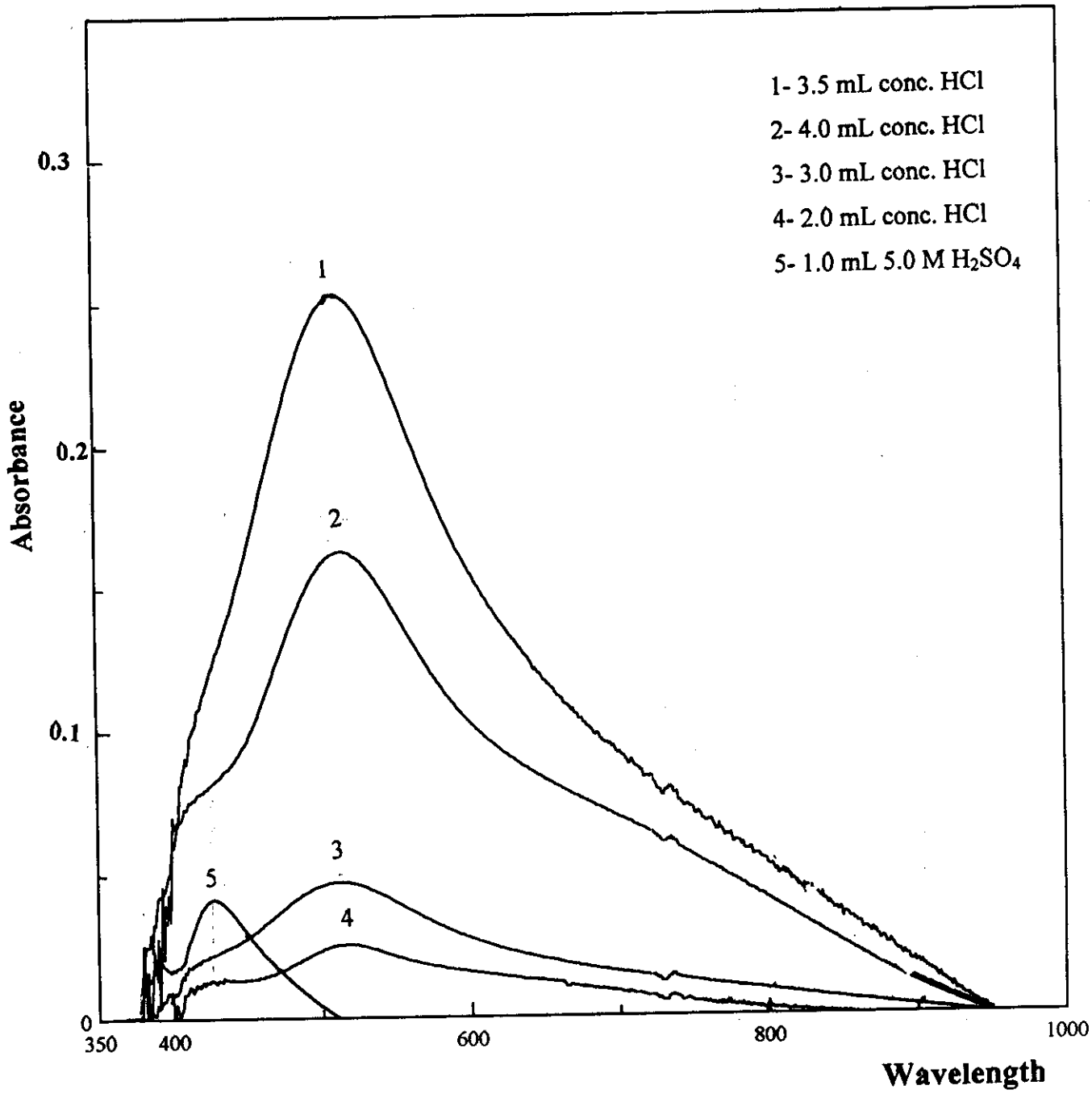


Fig. (29): Absorption spectra of 50 μg mL^{-1} of Vanc.Cl using 5×10^{-2} M $K_4[Fe(CN)_6]$ at different acid media.

The careful investigation of these studies showed that the optimum acid media recommended for subsequent studies of the coloured species between potassium ferrocyanide and Amik.SO_4, Neorm.SO_4 and Tobr.SO_4 was phosphoric acid, whereas for Vanc.Cl, the optimum acid media was the hydrochloric acid.

In order to investigate the optimum acid concentration facilitating the oxidation process, different amounts of each of concentrated phosphoric and 2.0 M hydrochloric acid were added to 50 μg mL^{-1} for Amik.SO_4 and Vanc.Cl and 6.0 μg $mL^{-1}$for Neom.SO_4 and Tobr.SO_4. The absorbance was then measured against a blank solution prepared by the same manner except the drug solution. The results obtained indicated that 1.0 mL of concentrated phosphoric acid is sufficient for complete colour development in case of Amik.SO_4 and Neom.SO_4 although 3.0 mL is needed for complete colour intensity in case of Tobr.SO_4. For Vanc.Cl, 3.5 mL of 2.0 M hydrochloric acid is enough for complete colour production. Increase volume and concentration will affect the colour intensity and so decrease the sensitivity. A representative figure for the effect of acid concentration is shown in [Fig. (30)].

3.4.2. Effect of reagent concentration

The effect of varying concentration of $K_4[Fe(CN)_6]$ on the absorbance of the formed colour species was obtained using 50 μg mL^{-1} in case of Amik.SO_4 and Vanc.Cl and 6.0 μg mL^{-1} of Neom.SO_4 and Tobr.SO_4. The most pronounced effect was obtained by adding (0.5-5.0 mL) of $5x10^{-2}$ M reagent solution. The obtained

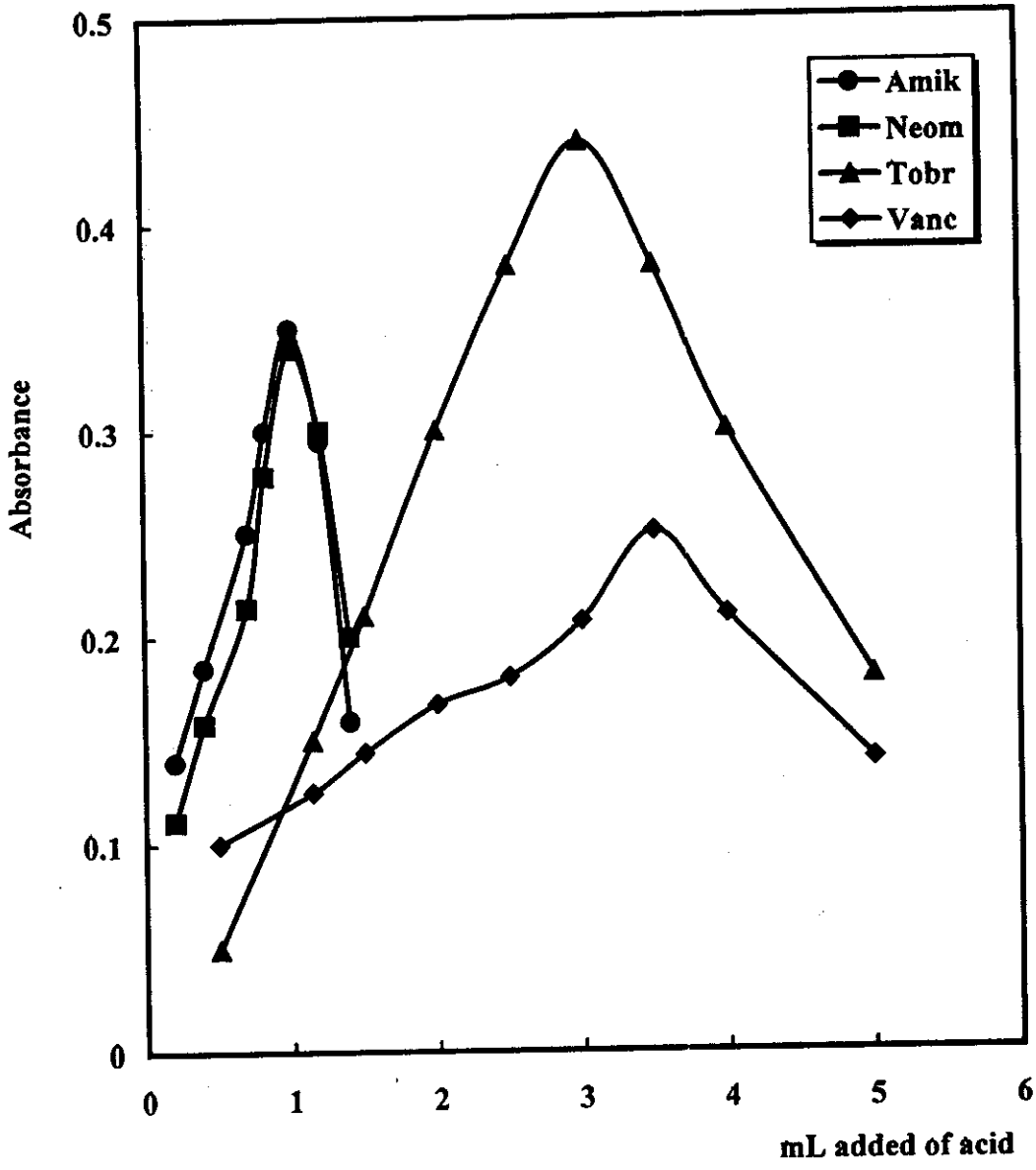


Fig. (30): Effect of mL added of concentrated H_3PO_4 acid on the absorbance of 1.5 x 10^{-2} M $K_4[Fe(CN)_6]$ and 50 µg mL^{-1} Amik.SO_4, and 6.0 µg mL^{-1} for Tobr.SO_4 or Neom.SO_4. For 50 µg mL^{-1} Vanc.Cl 2.0 M Hclwas used.

results indicated that the absorbance of ion pair complex is small using lower volume of reagent from 0.5-1.5 mL. From 2.0 to 2.5 mL absorbance increase until it reached maximally on using 3.0 mL of $5x10^{-2}$ M of $K_4[Fe(CN)_6]$ for all studied drugs. Increasing the reagent concentration a decrease in the absorbance was observed which may be due to increasing the intensity of the blank or dissociation of the sample containing. All subsequent studies were made using 3.0 mL of $5x10^{-2}$ reagent solution. A representative illustration of this effect was shown in [Fig. (31)].

3.4. 3. Effect of time and temperature

The time required for complete colour intensity of the formed coloured species between $K_4[Fe(CN)_6]$ and each aminoglycoside drugs was investigated. Allowing the reactants to stand for different time intervals, it was observed that after 3.0 hrs of mixing, the colour start to appear, increase and reach its maximum after 5.0 hrs. To overcome this problem heating the mixture on a water bath of different temperature for different time interval indicated that raising the temperature decrease the time of complete colour formation. The effect of the temperature was also studied by heating the sample and the blank solution. Raising the temperature increasing the absorbance and decreasing the time of complete colour development until 80, 80, 65 and 70°C which is the optimum temperature for Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl, respectively. Heating at optimum temperature for different time to achieve maximum colour development was investigated. [Fig. (32)] illustrated the effect of time at the optimum temperature, indicating that heating for

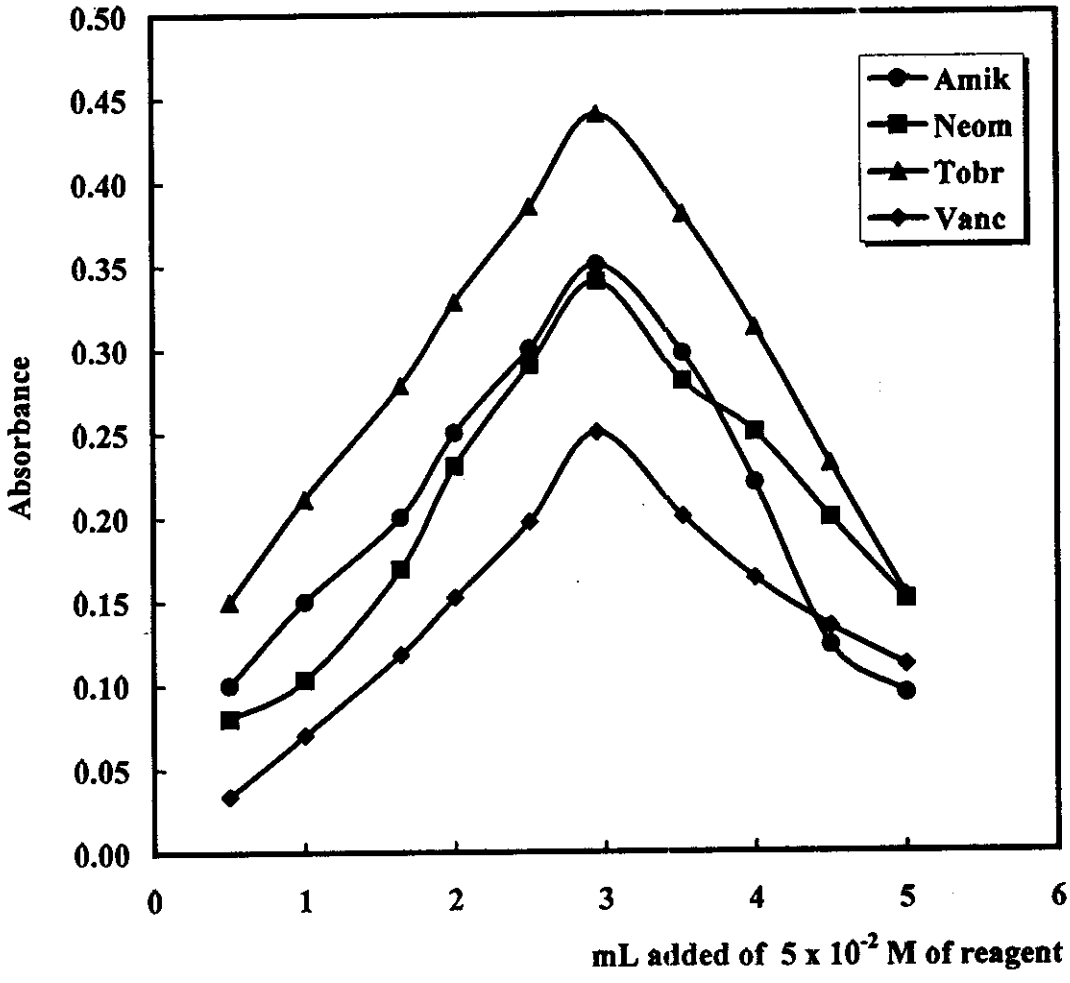


Fig. (31): Effect of reagent concentration on the absorbance of 50 μg mL^{-1} for Amik.SO_4 or Vanc.Cl and 6.0 μg mL^{-1} for Neom.SO_4 and Tobr.SO_4 solution.

15, 10, 20 and 15 min at 80, 80, 65 and 70°C for Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl, respectively.

3.4.4. Effect of solvent and sequences of addition

The effectiveness of various organic solvents for the determination of the studied drugs was investigated such as ethanol, methanol, acetone, dioxane and water. All organic solvents were unsuitable owing to the limiting solubility of the complex in these solvents. All sequences of addition gave the same results for complete colour intensity.

3.4.5. Molecular ratio of the formed complex

In order to investigate the molecular ratio of the complexes formed between the studied drugs and ferriferrocyanide at the selected conditions, the molar ratio[55] and continuous variation [56,57], methods were carried out. The results indicated that the molecular ratio of the reagent to Amik.SO_4 is 2:3, to Neom.SO_4 is 1:3, to Tobr.SO_4 is 5:3 and to Vanc.Cl is 1:3. The shape of the curves indicated that; the ion pair complex formed were labile, as shown in [Fig. (33)]. Hence, a large excess of reagent must always be used to enhance the formation of the complex.

$$3[Amik.2SO_4] + 2Fe_4[Fe(CN)_6]_3 \rightarrow \{Amik\}_3\{Fe_2[Fe\ (CN)_6]_3\}_2 + 2Fe_2(SO_4)_3$$

$$3[Neom.SO_4] + Fe_4[Fe(CN)_6]_3 \rightarrow \{Neom\}_3\{Fe_2[Fe(CN)_6]_3\} + Fe_2(SO_4)_3$$

$$3(Tobr)_2(SO_4)_5 + 5Fe_4[Fe(CN)_6]_3 \rightarrow \{Tobr\}_6\{Fe_2[Fe(CN)_6]_3\}_5 + 5Fe_2(SO_4)_3$$

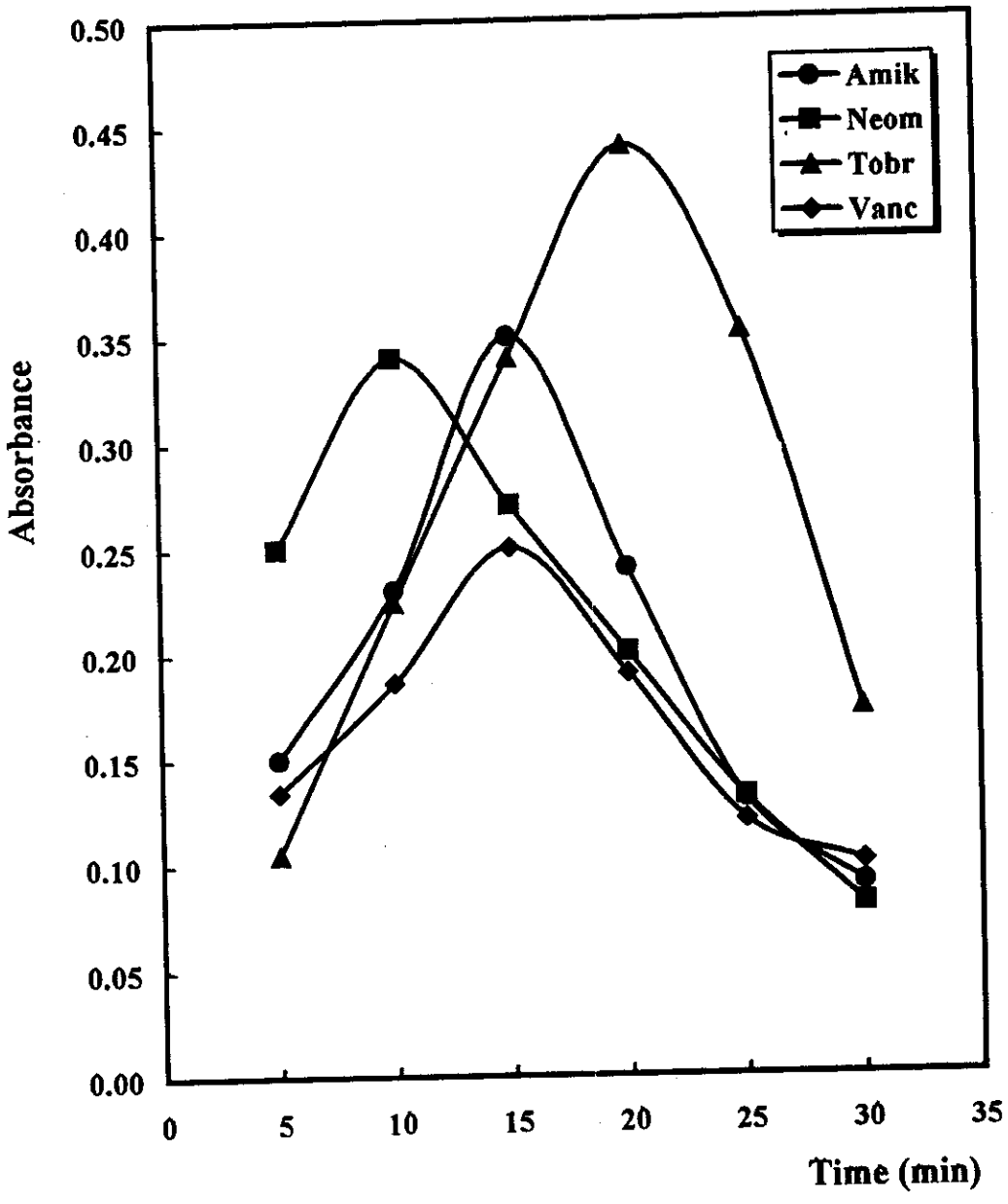


Fig. (32): Effect of time on the colored product formed between 5 x 10^{-2} M reagent and 50 µg mL^{-1} of Amik.SO_4 and Vanc.Cl or 6.0 µg mL^{-1} of Neom.SO_4 and Tobr.SO_4, respectively.

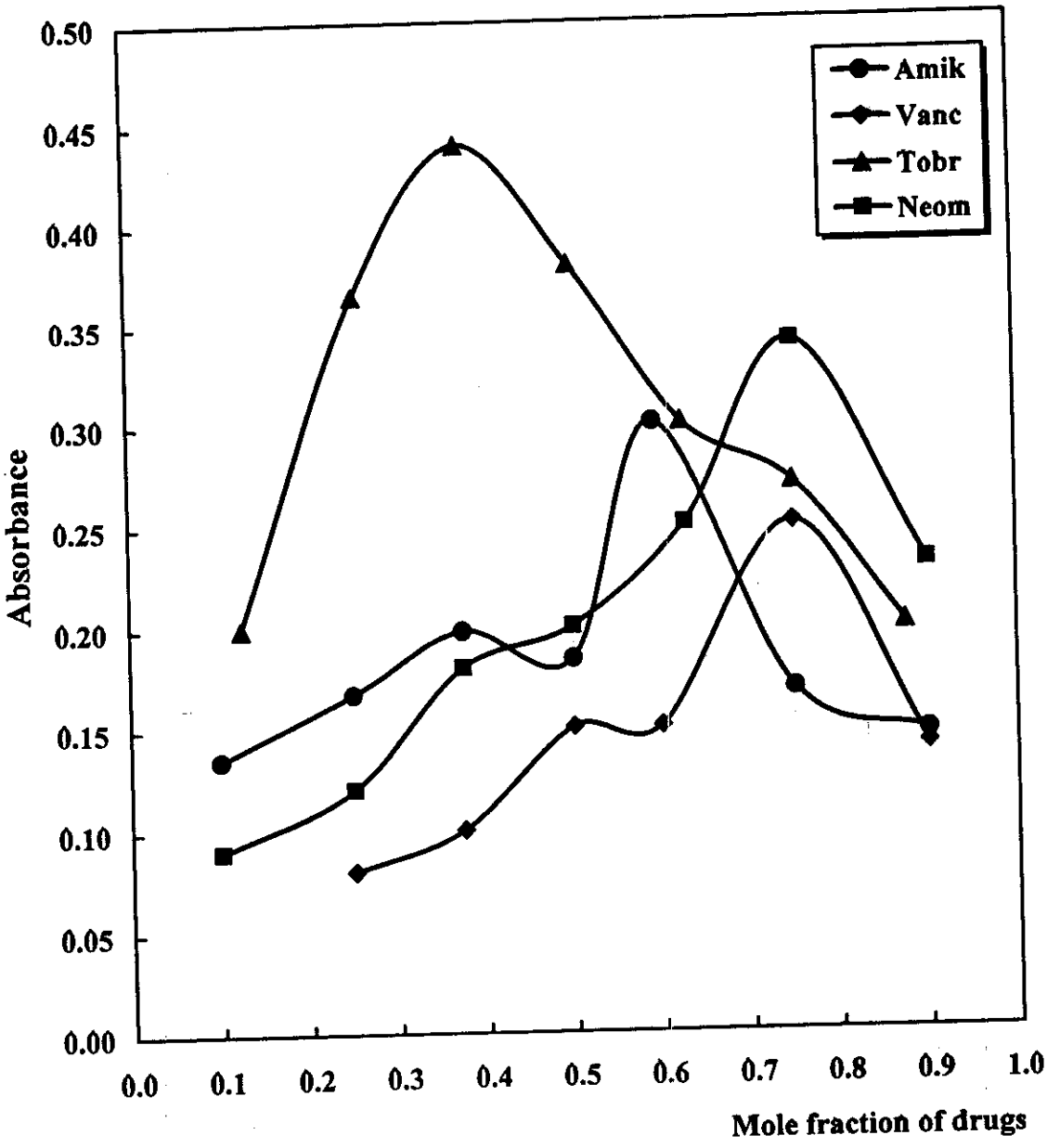


Fig. (33): Continuous variation method for Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl using 5 x 10^{-2} M $K_4[Fe(CN)_6]$.

$$3[Vanc.Cl] + Fe_4[Fe(CN)_6]_3 \rightarrow \{Vanc\}_3\{Fe_3[Fe(CN)_6]_3\} + FeCl_3$$

The stability constant of the complexes were calculated by using data of the molar ratio and Job's continuous variation methods applying the Harvey and Manning equation [67]. The results indicated that the logarithmic stability constants were found to be, and for Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl, respectively.

3.4.6. Statistical analysis

The statistical analysis of each variable was made showing the sample mean ($\bar{X}$) and the standard deviation (s). These values are calculated according to the following equation:

Mean value $(\bar{X}) = \sum_i X_i / n$

Standard deviation (S)= $\sqrt{\sum_i (X_i - \bar{X})^2 / (n-1)}$

Where n = Number of observations

Σ = Summation

X_i = Individual observations

The regression equation A = a + bc

Where A = Absorbance

C = Concentration in $\mu g\ mL^{-1}$

Slope and regression coefficient were calculated using the following formulae

Slope (b)= $$\frac{\sum_i[(X_i-\bar{X})(Y_i-\bar{Y})]}{\sum_i(X_i-\bar{X})^2}$$

Regression coefficient (r) = $$\frac{\sum_i[(X_i-\bar{X})(Y_i-\bar{Y})]}{\left\{\left[\sum_i(X_i-\bar{X})^2\right]\left[\sum_i(Y_i-\bar{Y})^2\right]\right\}^{1/2}}$$

Standard deviation for the slope (S_b)= $$\frac{\left[\sum_i(Y_i-\bar{Y}_i)^2/n-2\right]^{1/2}}{\left\{\left[\sum_i(X_i-\bar{X})^2\right]\right\}^{1/2}}$$

Where the fitted Y-values (Y_i) are the points on the calculated regression line corresponding to the individual x-values.

Standard deviation of the intercept (S_a) = $$\left\{\frac{\sum_i(Y_i-\bar{Y}_i)^2}{n-2}\right\}^{1/2}\left\{\frac{\sum_i X_i^2}{n\sum_i(X_i-\bar{X})^2}\right\}^{1/2}$$

Relative standard deviation RSD = 100 $\overline{S/X}$

Relative error R.E. = $100\frac{\Delta\bar{X}}{\bar{X}}$

Where $\Delta\bar{X} = S.t/\sqrt{n}$

and t is the tabulated value for t-test at 95% confidence level for five degree of freedom.

3.4.7. Validity of Beer-Lambert law

Calibration graphs [Fig. (34,35)] were constructed using standard solutions of Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl. Under the optimum conditions, a linear relationship existed between the absorbance and concentration of the drug in the concentration rangs 5.0-130, 1.0-17, 0.2-7.0 and 5.0 to 65 $\mu g\ mL^{-1}$ of the above drugs, respectively, [Table (12)] were obtained. The correlation coefficient, slopes, intercepts, standard deviations of slopes and standard deviations of intercepts are calculated using the former equations. For more accurate results, Ringbom optimum concentration ranges were determined by plotting log [Drug], concentration of the drug in $\mu g\ mL^{-1}$, against percent transmittance [Fig. (36,37)] and the linear portion of the S-shaped curve gave an accurate range of analysis [Table (12)]. The mean molar absorptivity and Sandell sensitivity are calculated from Beer-Lambert law as recorded in [Table (12)], while a representative curve on the validity of Beer-Lambert law for Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl are shown in [Fig. (34,35)]. The detection and quantification limits were calculated from the standard deviation of the absorbance measurements obtained from a series of 13 blank solutions for each procedure. The limits of detection (K = 3) and of quantification (K = 10) of the method were established according to the IUPAC definitions ($C_l = KS_o/s$, where C_l is the limit of detection, S_o is the standard error of blank determination, s is the slope of the standard curve and K is the constant related to the confidence interval [68].

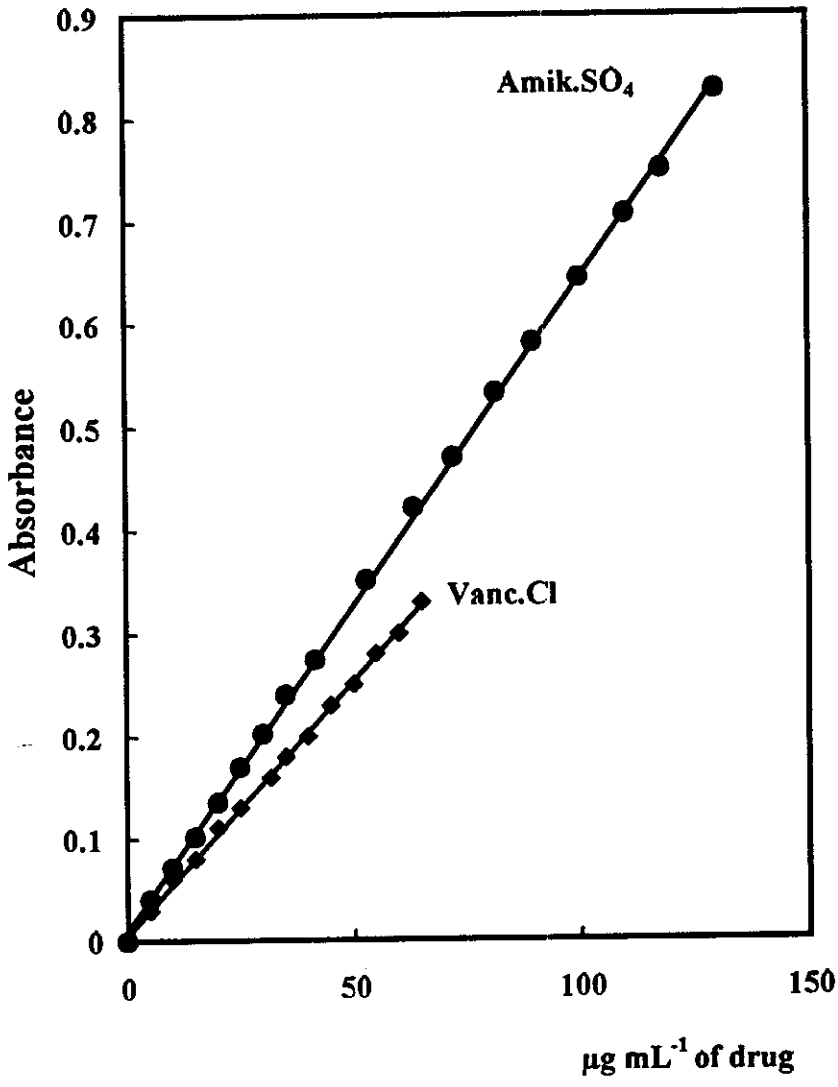


Fig. (34): Validation of Beer's law for Amik.SO_4 and Vanc.Cl complexes using 3.0 mL of 5 x 10^{-2} M $k_4[Fe(CN)_6]$.

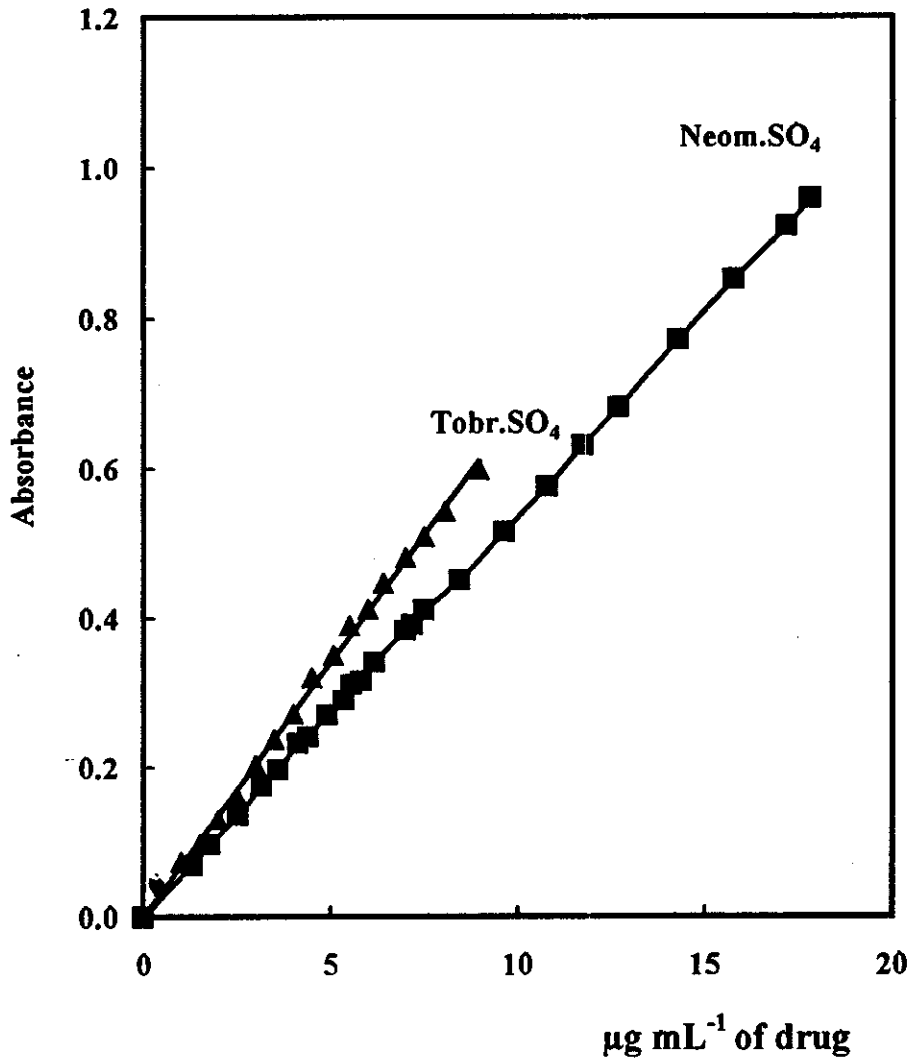


Fig. (35): Validation of Beer's law for Neom.SO_4 and Tobr.SO_4 complexes using 3.0 mL of 5 x 10^{-2} M $K_4[Fe(CN)_6]$.

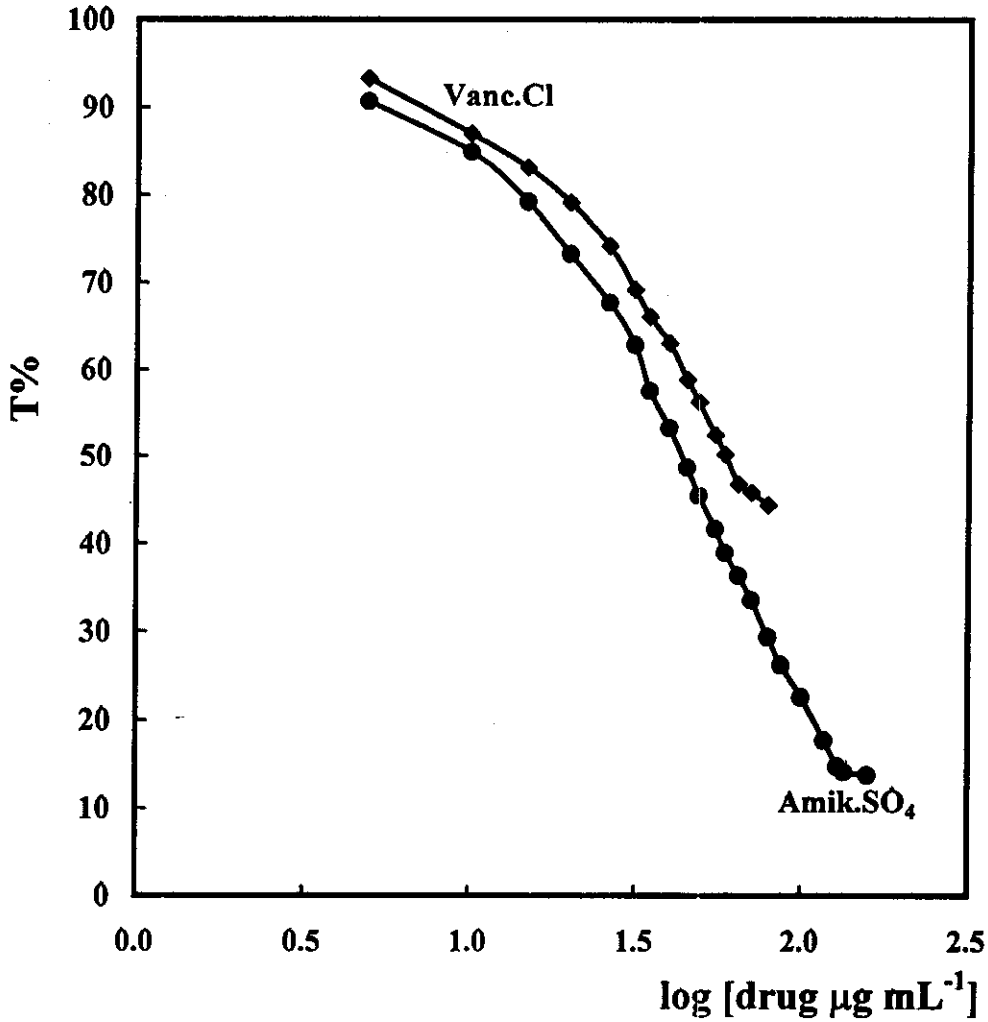


Fig. (36): Ringbom plots for Amik.SO_4 and Vanc.Cl using 5 x 10^{-2} M of $K_4[Fe(CN)_6]$.

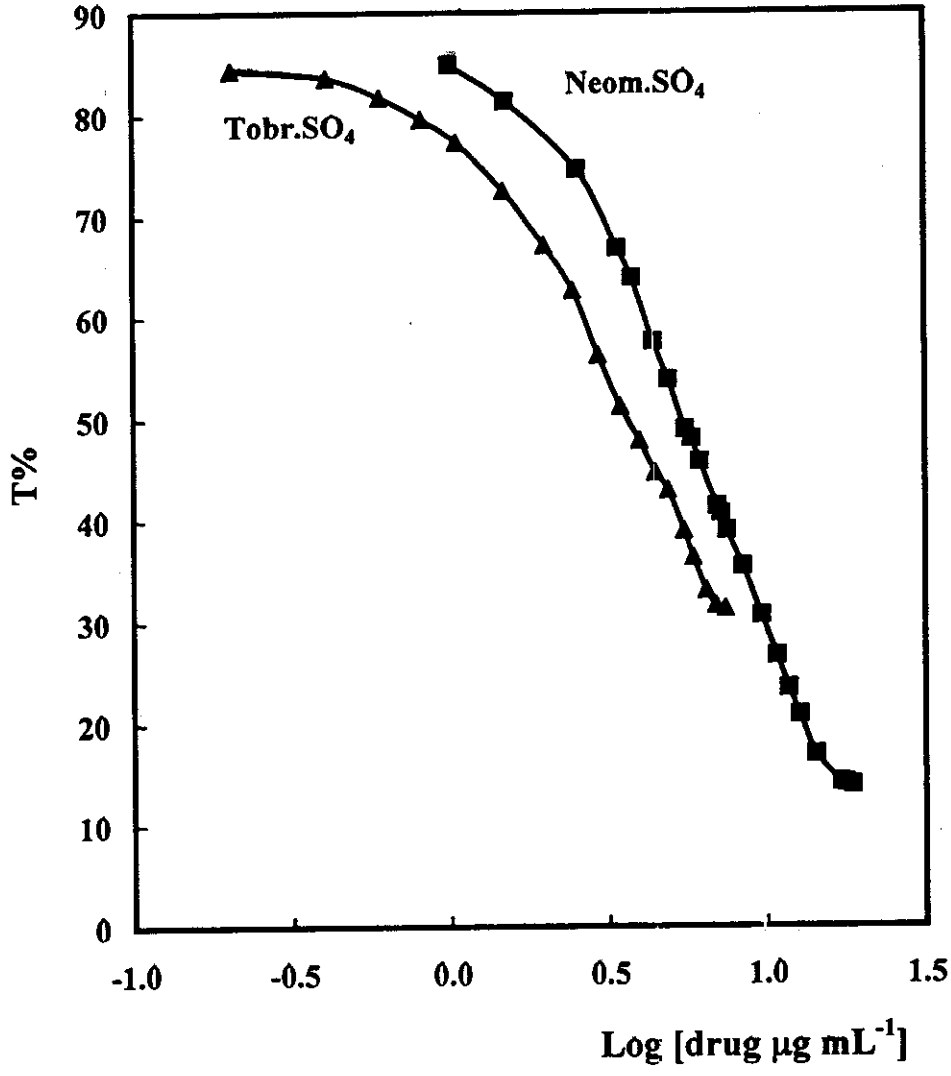


Fig. (37): Ringbom plots for Neom.SO_4 and Tobr.SO_4 using 5 x 10^{-2} M K_4[Fe $(CN)_6$].

3.4.8. Accuracy and Precision [69]

In order to determine the accuracy and precision of the proposed methods, solutions containing five different concentrations of the studied drugs were prepared and analysed in six replicates. The analytical results obtained from this investigation are summarized in [Table (13)]. The percentage standard deviations and the percentage range of error at 95 % confidence level were calculated. The results can be considered to be very satisfactory, at least for the level of concentrations examined.

3.4.9. Interference

A systematic quantitative study was undertaken by measuring the absorbance of solutions containing 50 μg mL^{-1} for Amik.SO_4 and Vanc.Cl or 6.0 μg mL^{-1} for Neom.SO_4, and Tobr.SO_4, respectively, using the recommended methods. No significant interference (less than ± 3.0 % in absorbance is considered non-interference) were observed from common additives and excipients usually presents in pharmaceutical formulations such as, lactose, fractose, glucose, calcium hydrogen phosphate, magnesium stearate and starch. Also there was no interference from common degradation products results from thermal and hydrolytic degradation of the studied drugs.

3.4.10. Analytical applications

The validity of the proposed procedure by determining Amik.SO_4, Neom.SO_4, Tobr.SO_4 and Vanc.Cl in their dosage forms obtained from local companies as mention before and urine sample were examined. The concentration of the studied drugs in dosage

forms and in urine samples was calculated from the appropriate calibration graphs. There was no shift in the absorption maximum nor absorbance due to the presence of other constituent on the dosage forms. The results are compared with those obtained by applying the official methods [63,64]. The results obtained were compared statistically by the Student's t-test for accuracy and variance ratio F-test for precision with those obtained by the official methods on the sample of the same batch. The Student's t- values obtained at 95% confidence level and five degrees of freedom did not exceed the theoretical tabulated value indicating no significant difference between accuracy of the proposed and the official methods [Table (14)]. Also, the variance ratio F-test obtained at 95% confidence level and five degree of freedom did not exceed the theoretical tabulated value indicating no significant difference between precision of the proposed and the official methods as recorded in [Table (14)]. The accuracy and precision of the proposed methods for investigated drugs in urine samples are summarized in [Table (15)].

Table (12). Analytical characteristics of the coloured species using $K_4[Fe(CN)_6]$

Parameter	Drug			
	Amik.SO_4	**Neom.SO_4**	**Tobr.SO_4**	**Vanc.Cl**
Acid	H_3PO_4	H_3PO_4	H_3PO_4	2 M HCl
Volume of acid used/mL	1.0	1.0	3.0	3.5
Temperature /°C	80	80	65	70
Time/min	15	10	20	15
λ_{max} /(nm)	523	523	504	507
Beer's law limit / μg mL^{-1}	5.0 - 130	1.0 - 17	0.2- 7.0	5.0 – 65
Ringbom limits / μg mL^{-1}	10.0-126	1.4-16.2	0.3-6.85	7.0-62
Molar absorptivity / L mol^{-1} cm^{-1}	$4.73x10^4$	$5.13x10^4$	$1.27x10^5$	$7.10x10^3$
Sandell sensitivity / ng cm^{-2}	165	17.7	12	204
Detection limits / μg mL^{-1}	1.6	0.31	0.06	1.5
Quantification limits / μg mL^{-1}	4.95	0.98	0.19	4.8
Regression equation [a]				
Slope (b)	$6.05x10^{-3}$	$5.64x10^{-2}$	$8.25x10^{-2}$	$4.90x10^{-3}$
Standard deviation of slope (S_b)	$6.01x10^{-3}$	$4.56x10^{-3}$	$2.77x10^{-3}$	$0.68x10^{-3}$
Intercept (a)	$2.20x10^{-2}$	$-3.02x10^{-2}$	$1.38x10^{-3}$	$8.46x10^{-3}$
Standard deviation of intercept (S_a)	$5.95x\ 10^{-3}$	$4.12x10^{-3}$	$2.76x10^{-3}$	$0.66x10^{-3}$
Correlation coefficient (r)	0.9968	0.9881	0.9972	0.9995
Relative standard deviation/%	1.75	1.40	1.25	1.90

[a] $A = a + bC$, where C is the concentration in μg mL^{-1}.

Table (13). Evaluation of the accuracy and precision of the proposed procedures.

Drug	Taken mg	Recovery[a] %	RSD[a] %	RE %	Confidence limits
Amik.SO$_4$	25	98.0	0.36	0.66	24.5 ± 0.0078
	50	101.80	0.78	0.82	50.9 ± 0.0054
	75	101.07	0.48	0.51	75.8 ± 0.0097
	100	99.10	0.44	0.46	99.1 ± 0.0063
	125	99.2	0.38	0.40	124.0 ± 0.0051
Neom.SO$_4$	2.0	100.50	0.84	1.00	2.01 ± 0.0087
	4.0	99.75	0.85	0.90	3.99 ± 0.0106
	8.0	100.63	1.15	1.21	8.05 ± 0.0113
	12.0	100.83	1.03	1.09	12.10 ± 0.0091
	16.0	99.38	0.68	0.74	15.90 ± 0.0069
Tobr.SO$_4$	1.5	100.66	0.86	0.91	1.51 ± 0.0075
	3.0	99.67	1.13	1.20	2.99 ± 0.0083
	4.5	100.66	0.75	0.80	4.53 ± 0.0051
	6.0	100.83	1.00	1.05	6.05 ± 0.0108
	7.0	99.14	0.57	0.61	6.94 ± 0.0074
Vanc.Cl	12.0	99.58	0.47	0.51	11.95 ± 0.0091
	24.0	100.29	0.88	0.94	24.07 ± 0.0065
	36.0	99.72	0.70	0.74	35.90 ± 0.0175
	48.0	100.52	0.48	0.50	48.25 ± 0.0126
	60.0	100.33	0.76	0.80	60.20 ± 0.094

[a] : Average of six determinations

Table (14): Evaluation of accuracy and precision of the proposed and official methods for investigated drugs using potassium ferrocyanide in pharmaceutical preparation.

Dosage forms	Found* (mg)		R.S.D %	Recovery%		F ** ratio	t ** value
	O	P		O	P		
Likacin vial (500 mg) EIPICO	485.00	493.00	0.034	96.80	98.60	2.95	1.53
Amikin Vial (500 mg) Squibb	480.00	507.00	0.053	96.00	101.40	2.48	1.17
Amikin Vial (100 mg) Squibb	97.50	101.50	0.027	97.50	101.50	3.18	1.69
Neo-Cortef eye drop (5 mg/mL) EIPICO	4.94	5.09	0.072	98.80	101.80	3.50	1.83
Neo-Medrol eye drop (2.5 mg/mL) EIPICO	2.43	2.53	0.046	97.20	101.20	3.11	1.52
Neomycin tablet (500 mg) Memphis	515.00	495.00	0.067	103.00	99.00	2.79	1.42
Neomycin powder (125 mg)	121.00	127.00	0.058	96.80	101.60	2.81	1.37
Tobracin vial (20 mg) Memphis	18.30	20.20	0.040	91.50	101.00	2.35	1.08
Tobrin vial (20 mg) EIPICO	19.00	19.70	0.064	95.00	98.50	2.75	1.29
Tobrin eye drop (5 mg/mL) EIPICO	5.03	4.88	0.050	100.60	97.60	3.26	1.67

*: Average of six determinations

**: Theoretical values for t- and F- values for five degree of freedom and 95 % confidence limits are 2.57 and 5.05, respectively.

O: Official method

P: Proposed method

Table (15): Evaluation of accuracy and precision of the proposed and official methods for investigated drugs using potassium ferrocyanide in urine samples.

Urine samples	Added µg mL^{-1}	Found* µg mL^{-1}			
		Amik.SO_4	Neom.SO_4	Tobr.SO_4	Vanc.Cl
Urine 1	-	-	-	-	-
	3.0	2.78	2.97	3.02	2.70
	6.0	6.05	6.03	5.97	6.25
	12.0	11.95	11.92	-	11.95
	24.0	23.85	-	-	24.25
	48.0	48.40	-	-	47.5
	96.0	95.5	-	-	-
Urine 2	5.0	5.03	4.08	4.94	4.95
	10.0	9.90	10.15	-	9.92
	20.0	20.25	-	-	20.25
	40.0	39.80	-	-	39.7
	80.0	80.35	-	-	-

*: Average of six determinations