Resluts and discussion

3.1.1. Infrared spectra of free azo dye reagents

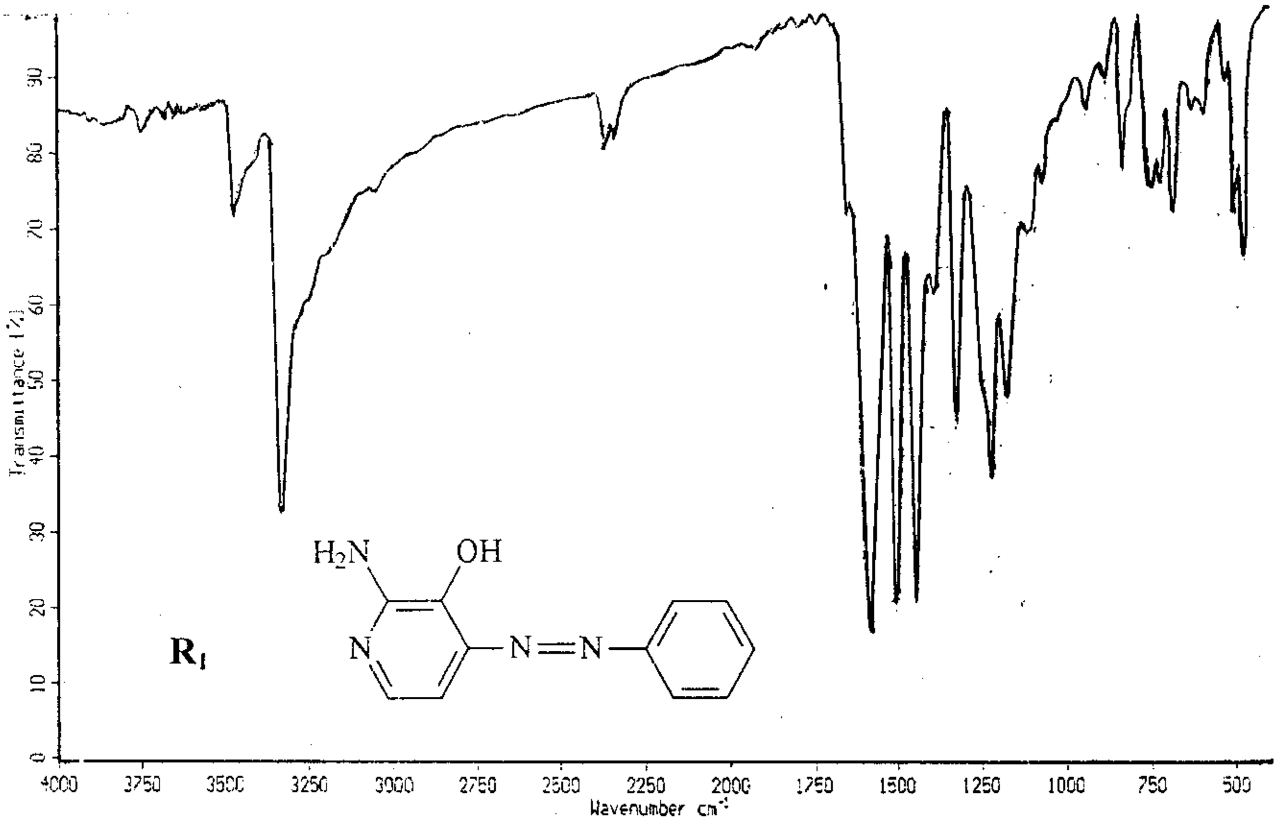
This part includes an attempt to obtaine the assignment for the importance and characteristatic bands in the IR-spectra of the reagents under investigation. The IR-spectra of the prepared azo dyes are recorded in Fig's. (1-4). The assignment of the important bands are given in Table (2).

3.1.1. Infrared spectra of R_1-R_4

In region of 3500-3300 cm^{-1}, the bands due to (OH) stretching vibrations are expected to appear. For the reagents (***R_1-R_4***) the ν_{OH} band appears as sharp absorption with medium intensity at frequency 3470, 3400, 3477 and 3420 cm^{-1} for (***R_1-R_4***), repectively. The ν_{NH} band appears at 3331, 3365, 3328 and 3336 cm^{-1} for (***R_1-R_4***), repectively. The ν_{CH} bands for aromatic system appears at 3049, 3063, 3010 and 3092 cm^{-1} for (***R_1-R_4***), repectively. The bands at 1625, 1664, 1587 and 1596 is due to C=C vibrations for (***R_1-R_4***), repectively.

The spectra in the range 1586-1434 cm^{-1} of the azo dye reagents are important, since the bands observed are attributed to $\nu_{C=N}$ and $\nu_{N=N}$ vibrations. The $\nu_{C=N}$ band is observed at 1586, 1566, 1509 and 1574 cm^{-1} while the $\nu_{N=N}$ is aboserved at 1448, 1434, 1453 and 1441 cm^{-1} for for (***R_1-R_4***), repectively. The appearance of the $\nu_{C=N}$ band for these reagents gives an indication that hydroxy azo$\longleftrightarrow$hydrazo equilibrium is taken place which confirmed by the apperance of weak band at 3261-3196 cm^{-1}of the stretching vibration of NH

Infrared Spectra of different azo dye



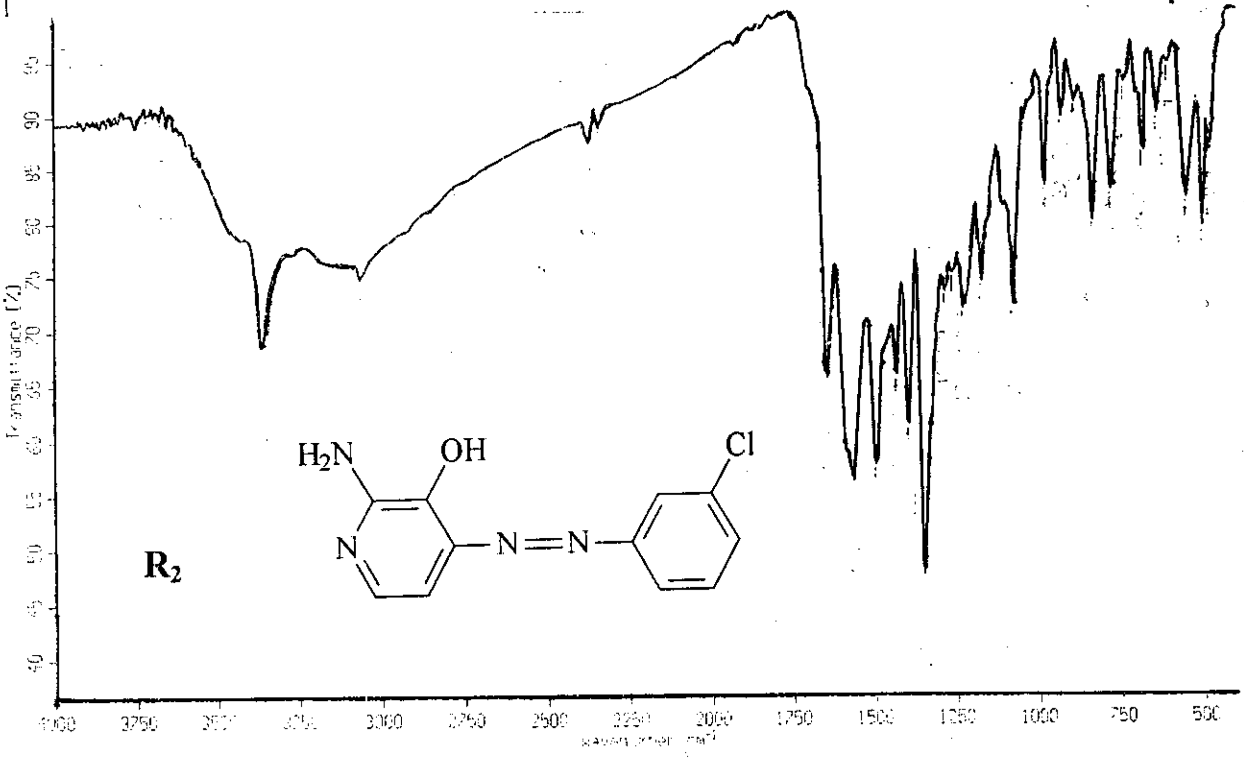
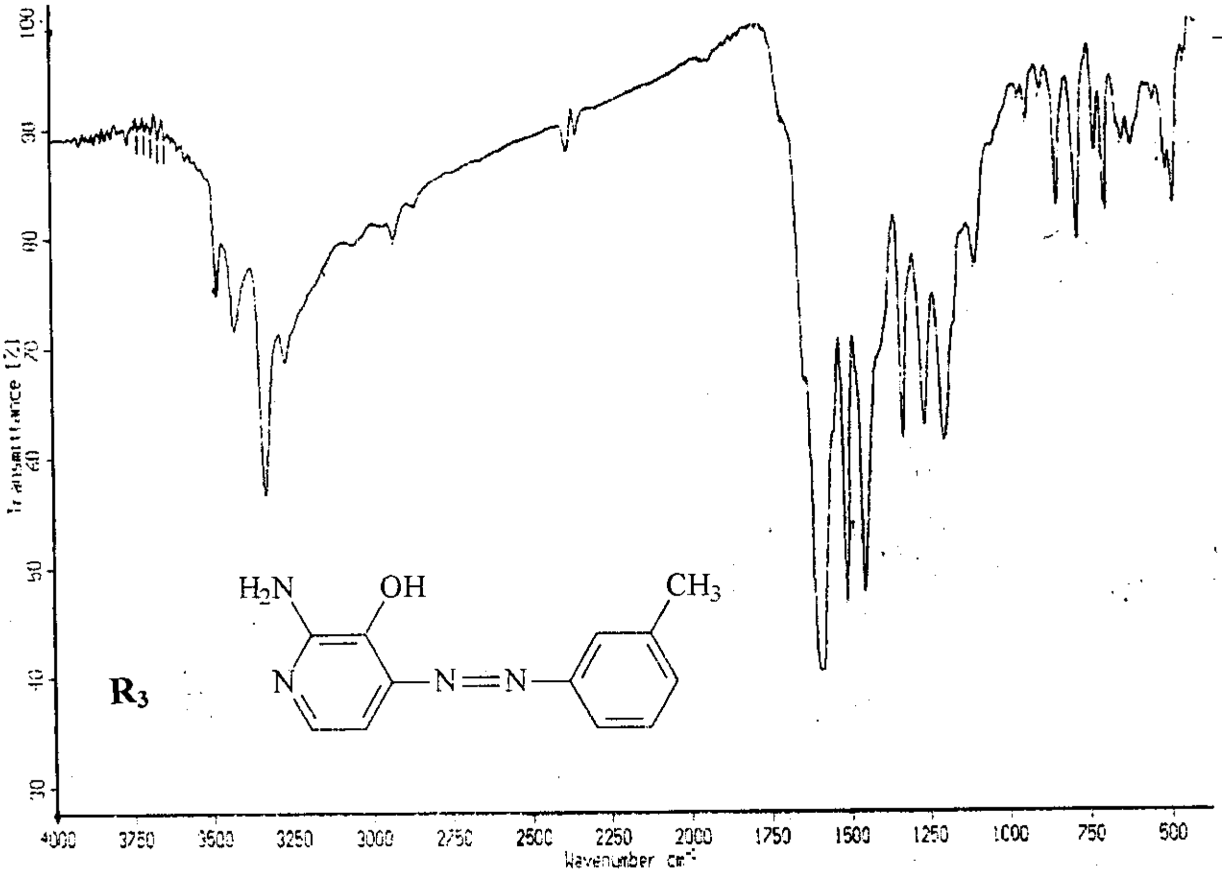


Fig. (1)

Infrared spectra of different azo dyes



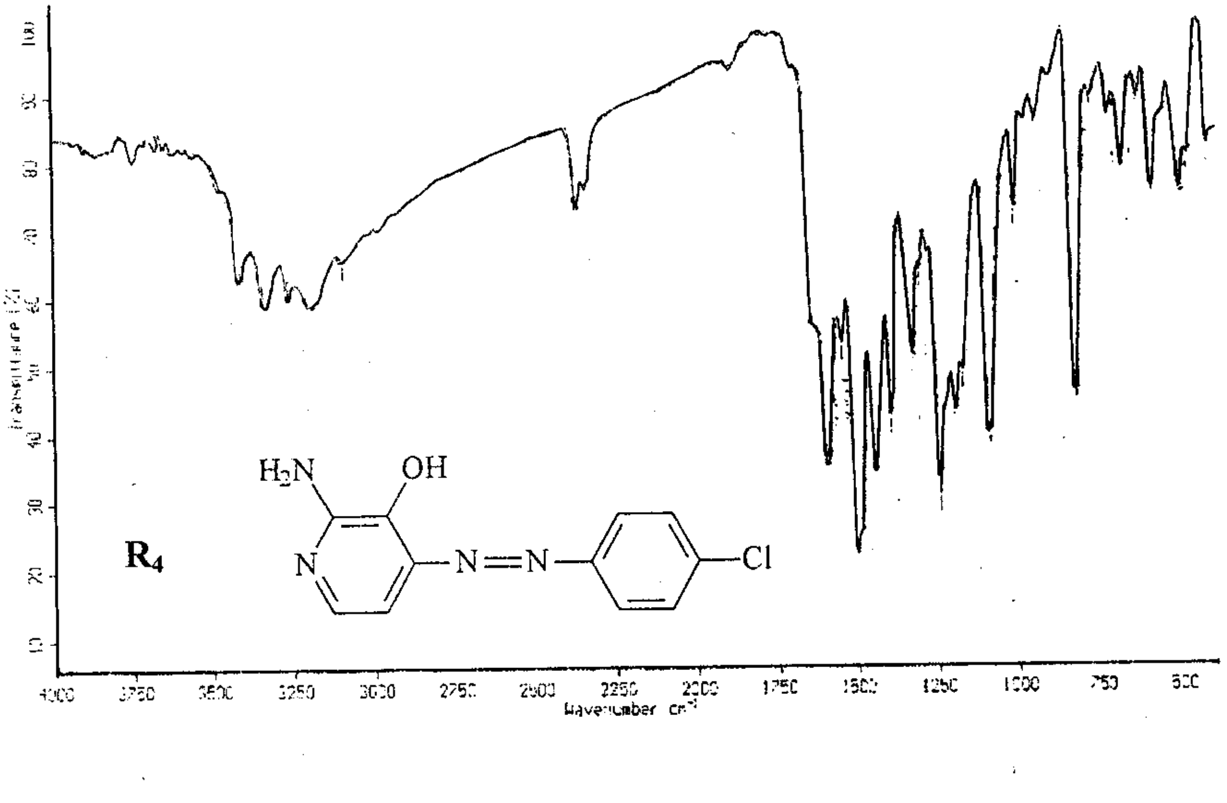
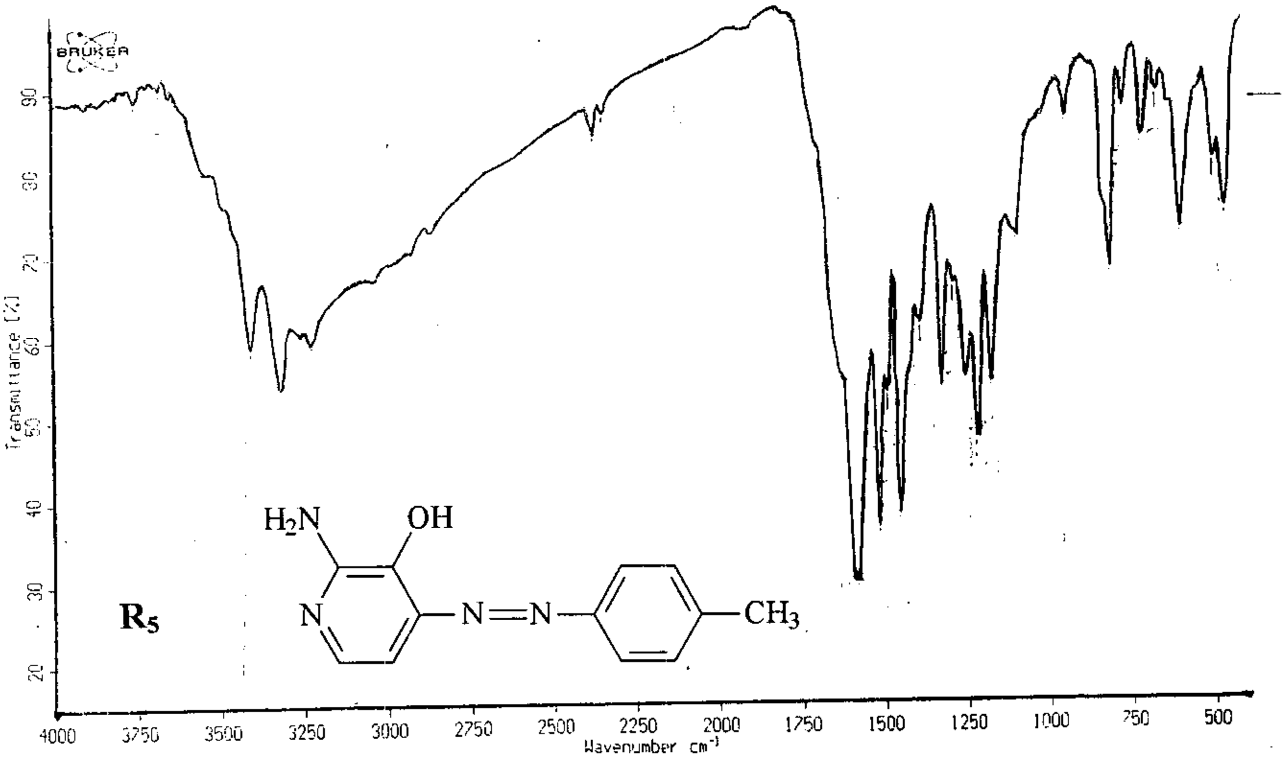


Fig. (2)

Infrared spectra of different azo dyes



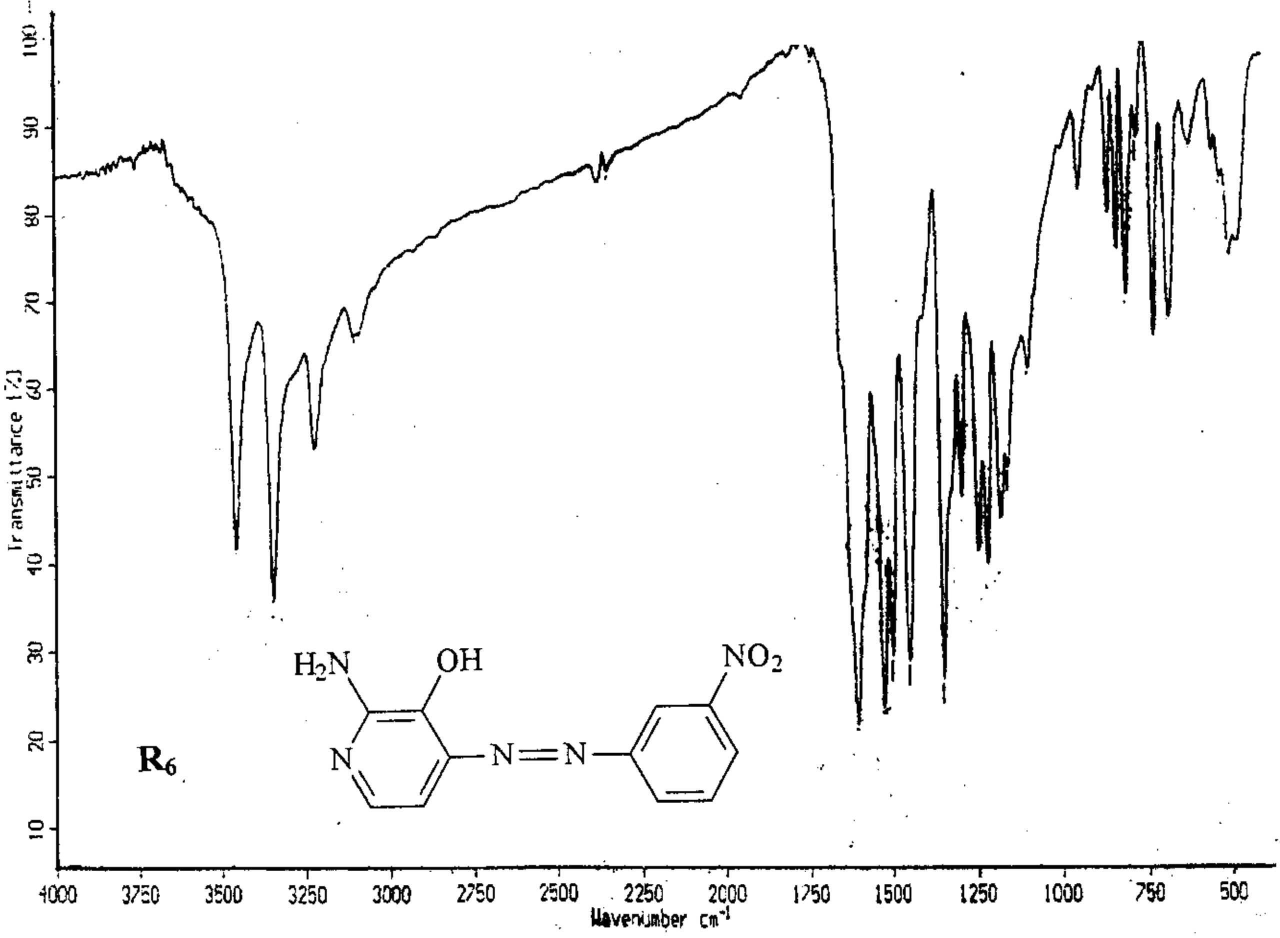
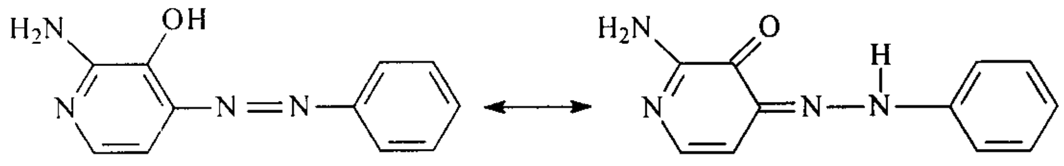


Fig. (3)

group resulting from hydroxy azo ⟷ hydrazo equilibrium and this can be represented as follows



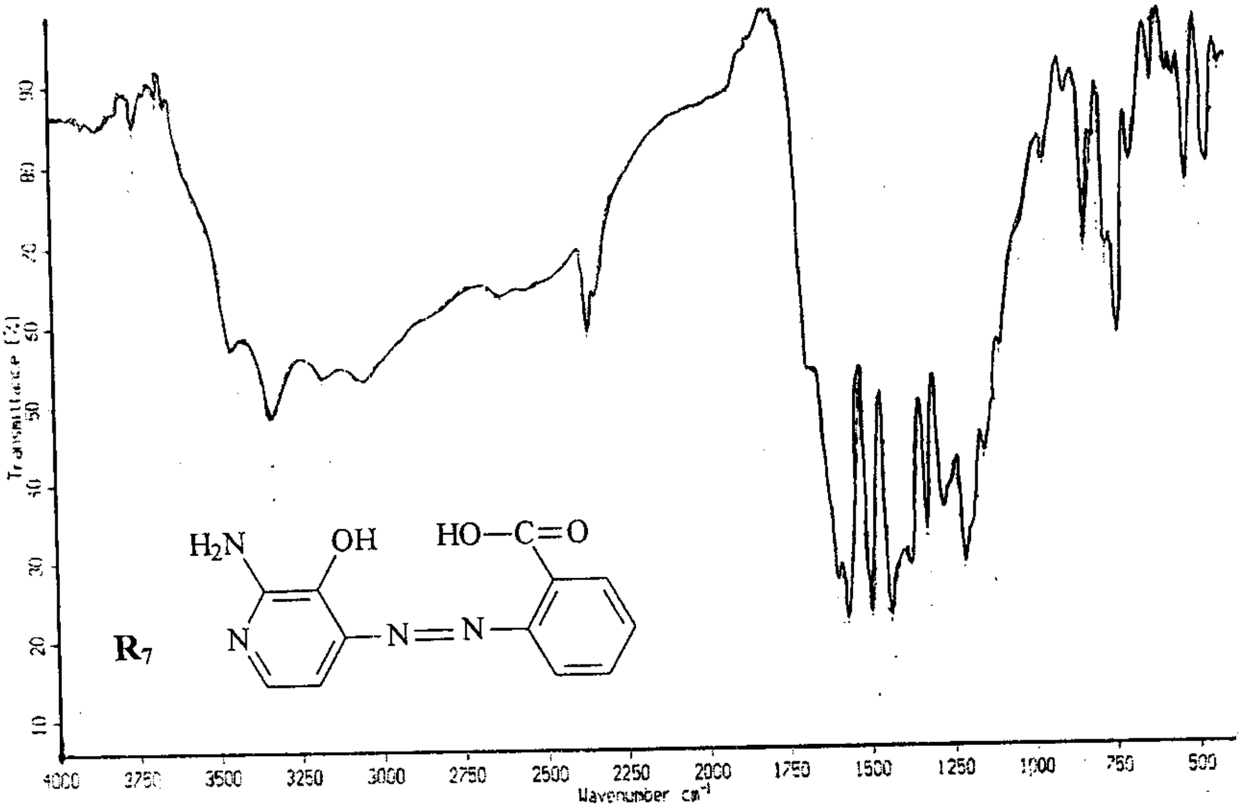
It is worthy to mention that the stretching vibration of the C=O group resulted from hydroxy azo ⟷ hydrazo equilibrium, taking place in azo compounds (***R_1-R_4***) which must be observed in this region is masked by the intense bands due to C=C ring vibrations.

3.1.2. Infrared spectra of R_5-R_8

For the reagents (***R_5-R_8***) the bands due to ν_{OH} vibration are appear at 3407, 3455, 3454 and 3436 cm^{-1}, while the bands attributed to ν_{NH} appear at 3319, 3345, 3333 and 3388 cm^{-1}, respectively. The aromatic C-H stretching vibration bands are observed at 3056, 3103, 3047 and 3064 cm^{-1} and the vibration C=C bands are appeared at 1584, 1607, 1601 and 1637 cm^{-1} for (***R_5-R_8***), respectively.

The stretching vibration bands of C=N appear at 1519, 1528, 1498 and 1495 cm^{-1} while the N=N bands appeared at 1456, 1450, 1439 and 1455 cm^{-1} for (***R_5-R_8***), respectively. The band due to ν_{NO} that presents in reagent ***R_6*** appears at 1528 cm^{-1}. The stretching vibration band of C=O of COOH group present in ***R_7*** and C=O of antipyaran ring of ***R_8*** appeared at 1601 and 1590 cm^{-1}, respectively. Also the apperance of weak band at 3222-3096 cm^{-1} is due to the stretching vibration of NH group resulting from hydroxy azo hydrazo equilibrium.

Infra red spectra of azo dye reagents



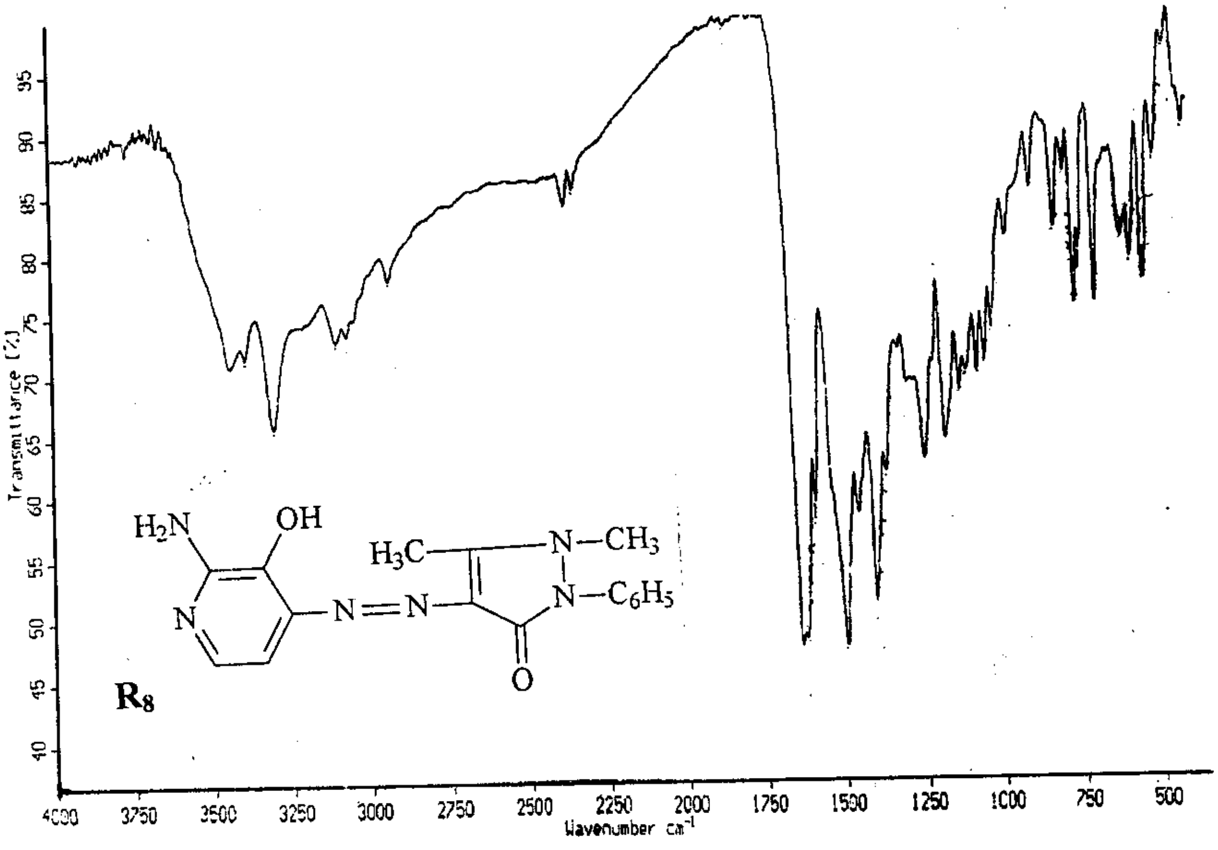


Fig. (4)

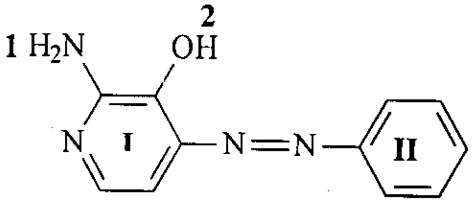
Table (2): The ir-vibrational frequencies (cm^{-1}) of some functional groups of reagents R_1-R_8.

Reagent	ν_{O-H}	ν_{N-H}	ν_{Ar-H}	$\nu_{C=C}$	$\nu_{C=N}$	$\nu_{N=N}$	ν_{C-H}	ν_{NO2}	$\nu_{C=O}$
R_1	3470	3331	3049	1652	1586	1448	----	----	----
R_2	3400	3365	3063	1646	1566	1434	----	----	----
R_3	3477	3328	3010	1587	1509	1453	2920	----	----
R_4	3420	3336	3092	1596	1547	1441	----	----	----
R_5	3407	3319	3056	1584	1519	1456	2859	----	----
R_6	3455	3345	3103	1607	1528	1450	----	1528	----
R_7	3454	3333	3047	1660	1498	1439	----	----	1601
R_8	3436	3388	3064	1637	1495	1455	2928	----	1590

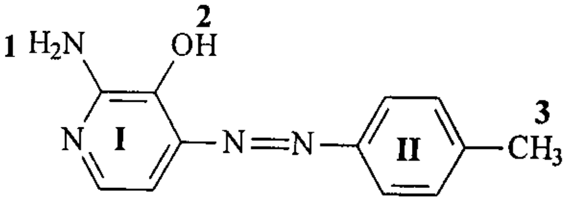
3.2. ^{1}H-NMR spectra of free azo dye reagents

A further support for the conclusion obtained from elemental analysis and IR spectra for the reagents under investigation is gained by a consideration of their ^{1}H-NMR spectra. The different types of hydrogen protons which are expected for some compounds under investigation can be formulated as follows

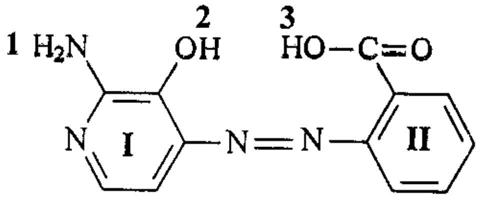
R$_1$



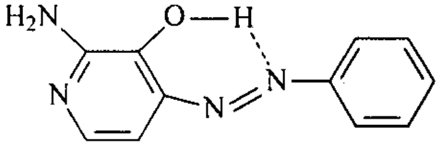
R$_5$



R$_7$



The different types of signals for hydrogens which are expected for some selected azo dye reagents ***R_1***, ***R_5*** and ***R_7*** are shown in Fig's. (5) and the chemical shift of different types of protons are recorded in Table (3). All signals observed take the integration value which gives evidence and helps to assign the signals. The signals lying at very upfield side 2.07-2.48 ppm are due to the hydrogen of OH group that are attached to pyridine ring. The values of magnetic resonance of hydrogen of OH group are shifted to upfield can be taken as an argument for the contribution of the OH group in an interamolecular hydrogen bond taking place as follow:



The signals lying at very downfield side 3.84-3.21 ppm for the same reagents are due to the hydrogens of NH_2 group. A new multiple signal at 1.23 ppm is due to the protons of CH_3 group of reagent ***R_5***. The signal at 8.25 ppm is due to the hydrogen of COOH group of reagent ***R_7***.The multi signals appear at 7.32-7.07 and 6.58-6.45 ppm are due to the protons of rings I and II respectively.

Proton magnetic resonance of reagents R_1, R_5 and R_7.

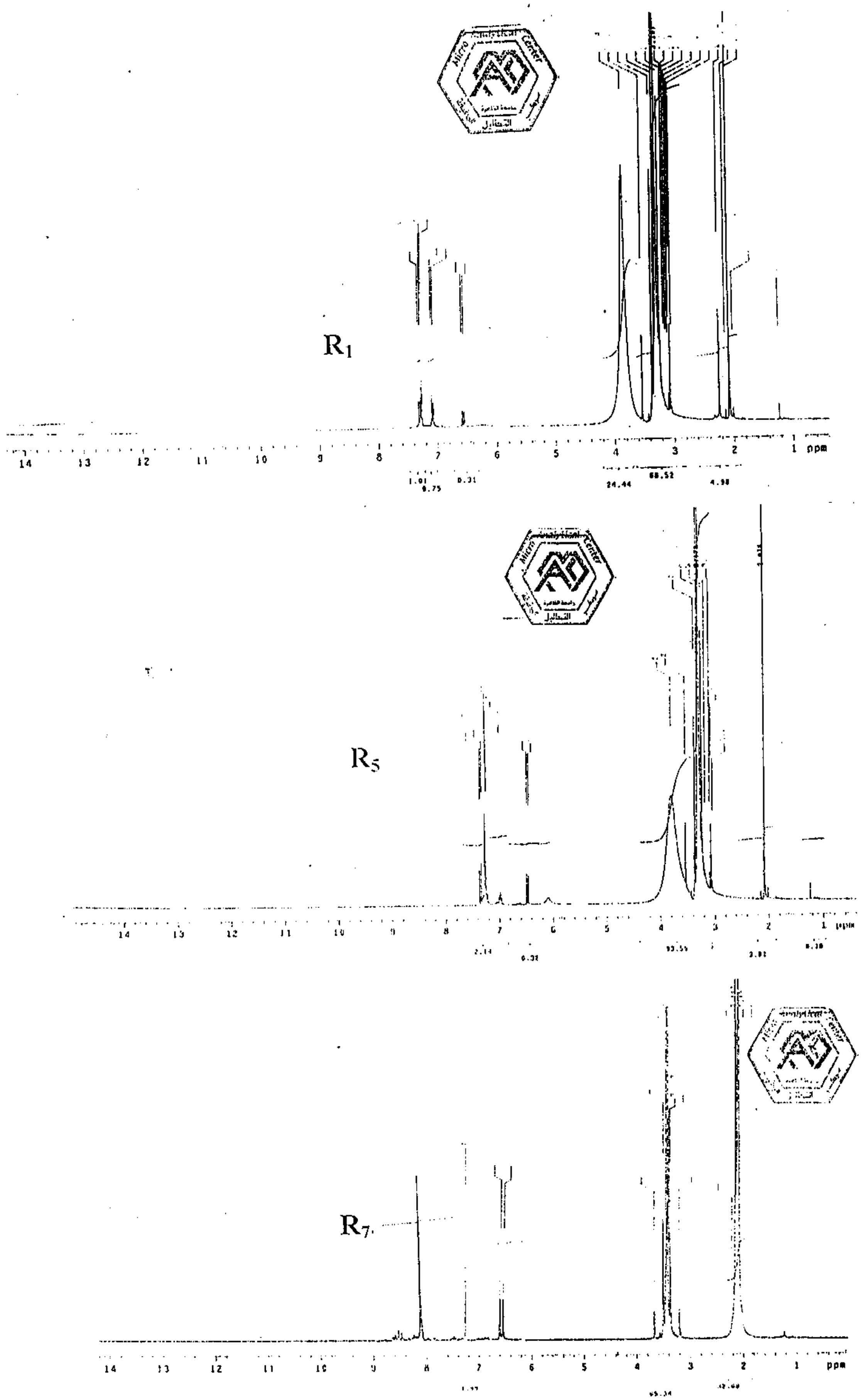


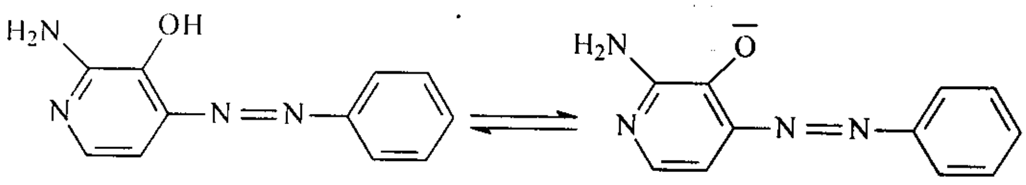
Fig. (5)

Table (3): Assignment and chemical shift (ppm) of different types of protons of some reagents

Compound	Chemical shift				
	(ppm) of protons			(ppm) of aromatic rings	
	H (1)	H (2)	H (3)	I	II
R_1	3.81	2.07	-----	7.37-7.25	6.49-6.45
R_5	3.84	2.84	1.23	7.32-7.07	6.58-6.55
R_7	3.67	2.14	8.25	7.52	6.50

3.3. The spectral behavior of reagents under consideration in buffer solutions and the determination of their acid constants

The acid ionization constants (pK_H) of the reagents under consideration are determined spectrophotometrically in universal buffer solutions covering the pH range 2.50 to11.50. The absorbation spectra of azo dyes in buffer soloutions of varying pH are recorded within the wavelength range 300-850 nm. The bands of some compounds are shifted in their position or showed variation in extinction whereas others exhibit a new band by increasing pH of the medium as shown in Fig's.(6-9). The results for all reagents indicated that the presence of acid-base equilibrium occurred between the non ionized and ionized species, this can be represented as follows:



Non ionized form　　　　Ionized form

The relation between pH and the absorbance of each reagent that shown in Fig's (6-9) give S-shape and the change in the absorbance with pH can be utilized for determination of the dissociation constants by using the following methods.

a) Half-height method(109):

This method is based on the fact that at the half-height of the absorbance-pH curve, the dissociated and undissociated species exist in equivalent quantities, thus

$$\mathbf{pK = pH} \qquad \text{at } A_{1/2}$$

where: $\mathbf{A_{1/2} = [(A_{max}-A_{min})/2]+ A_{min}}$

b) The modified limiting absorbance method(110)

This method has the advantage of eliminating any overlaps between the absorbance of the two forms and the pK_H is calculated by using the following equation

$$\mathbf{pH' = pK + \log y + \log [(A-A_{min}) / (A_{max}-A)]}$$

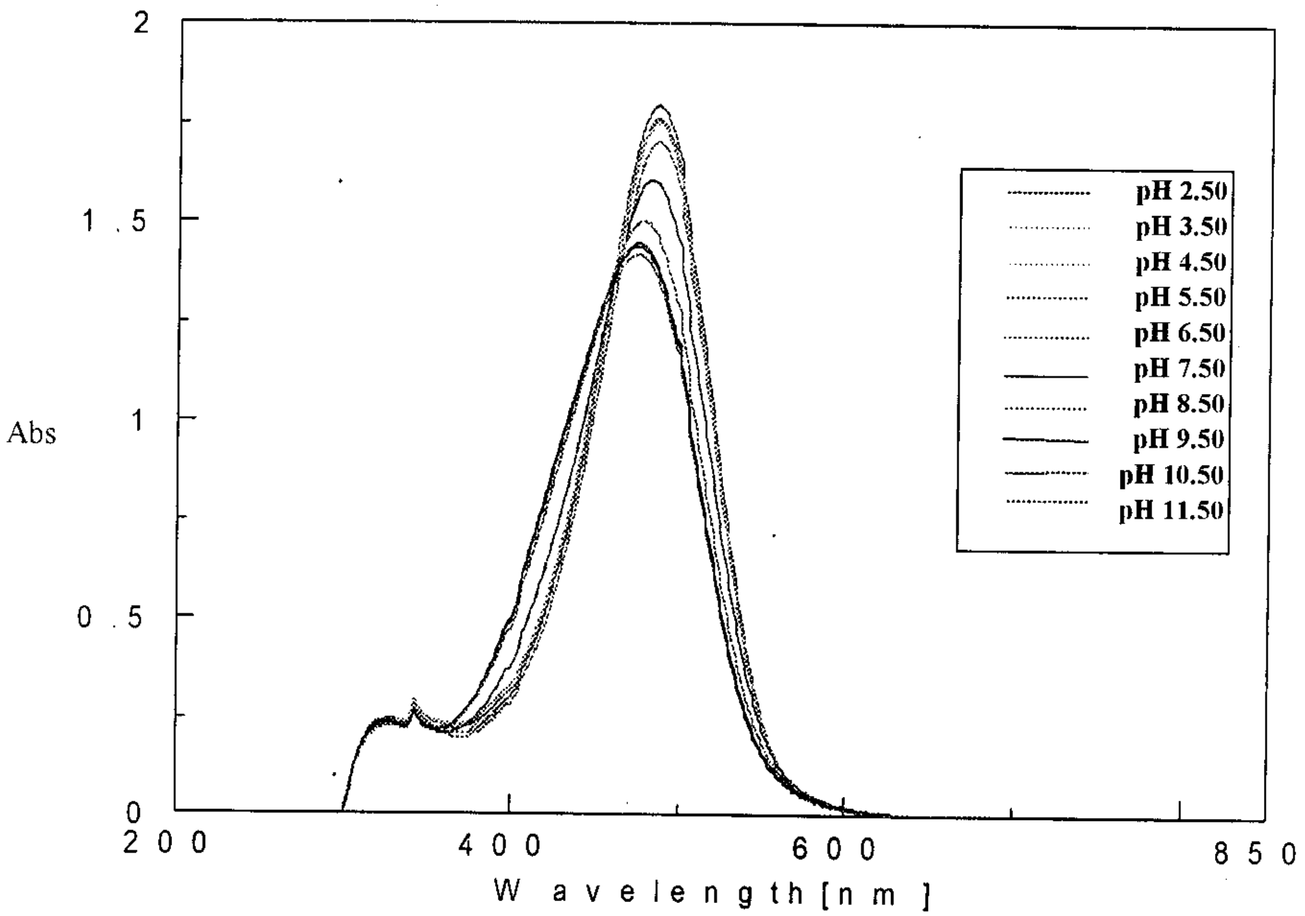
Where

A: the absorbance at a given pH value

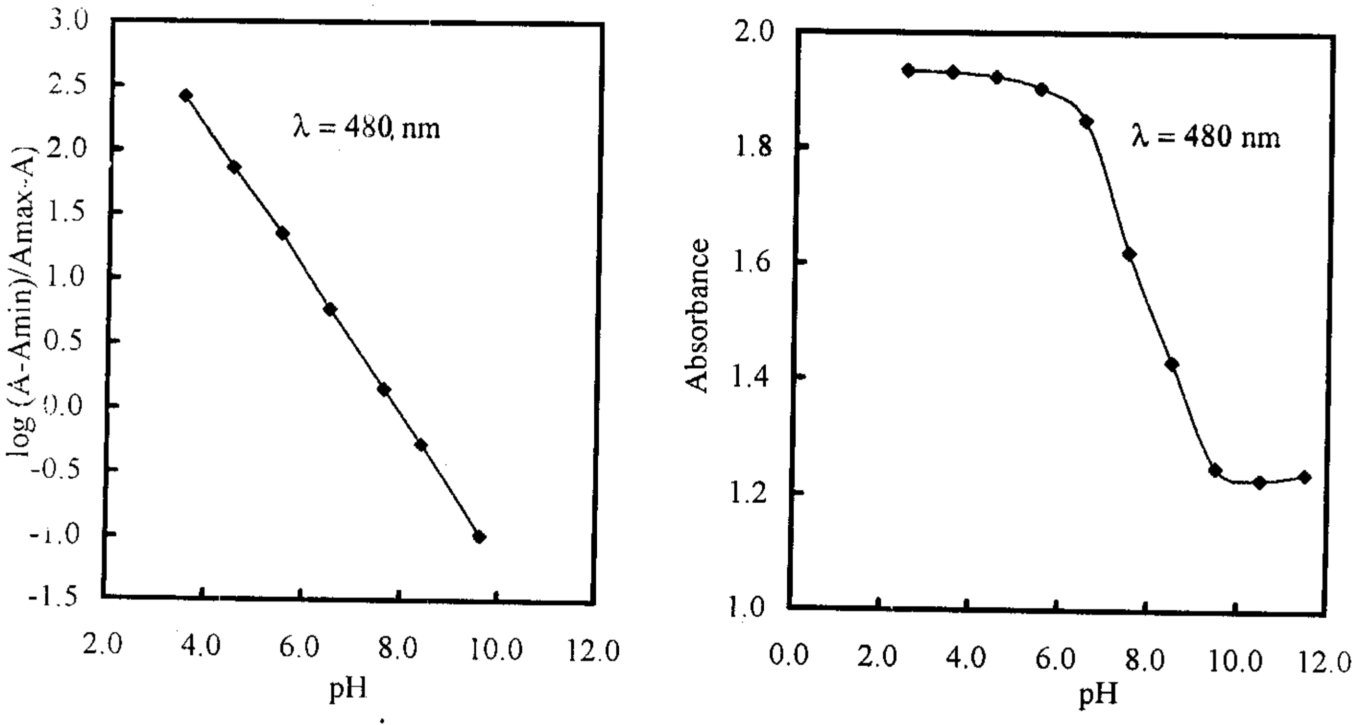
Y: the activity coeffecient of the ion present at equilibrium

$\mathbf{A_{maxi}}$, $\mathbf{A_{min}}$: are the absorbance corrsponding to the total concentration of neutral and ionized speccies liable to exist in solution, respectively.

The pK_H value can be evaluated by plotting log $[(A-A_{min}) / (A_{max}-A)]$ vs pH. The pK_H value thus corresponding to the pH value at zero log $[(A-A_{min}) / (A_{max}-A)]$. The ionization constants of different reagent are recorded in Table (4).



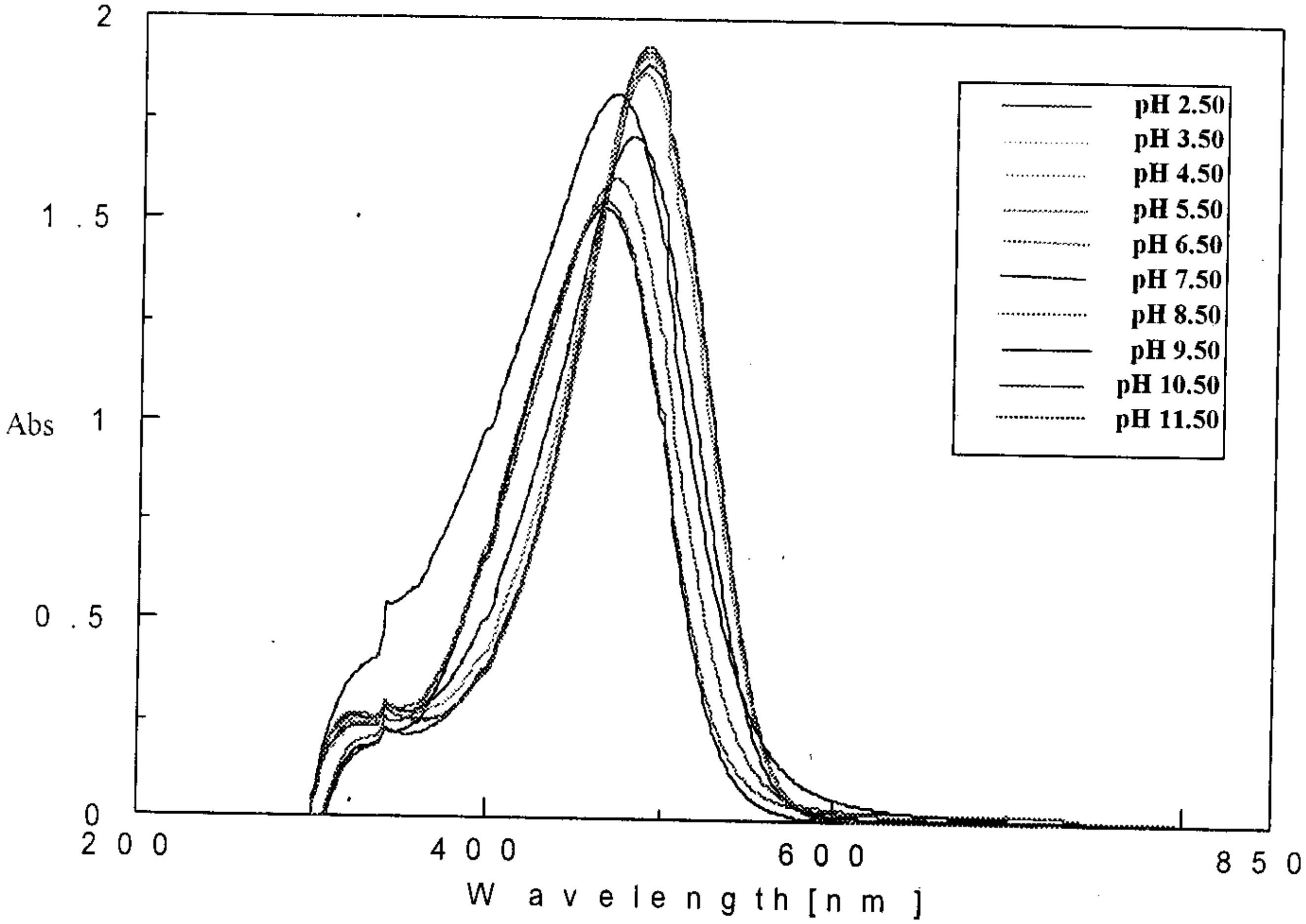
(a): Absorption spectra of (1.0 x 10^{-3} M) of reagent R_1 in buffer solutions of different pH values



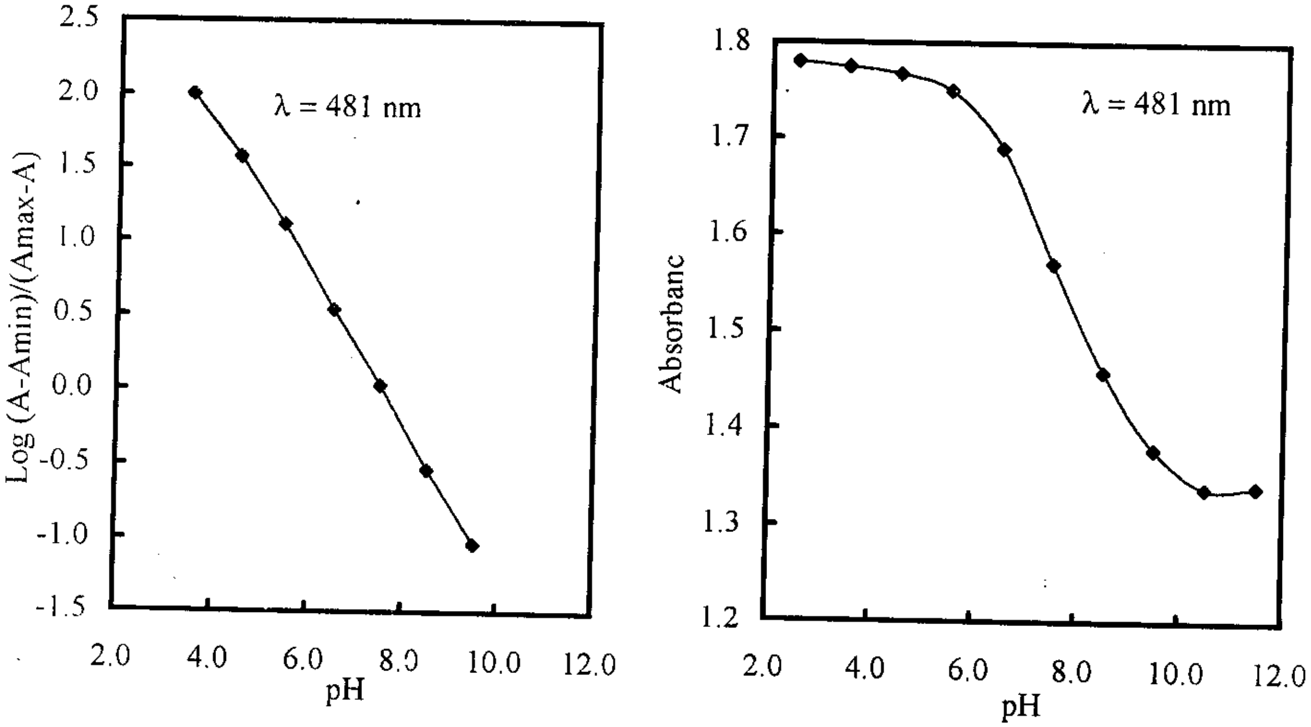
(c): The modified limiting method

(b): Half-height method

Fig. (6)



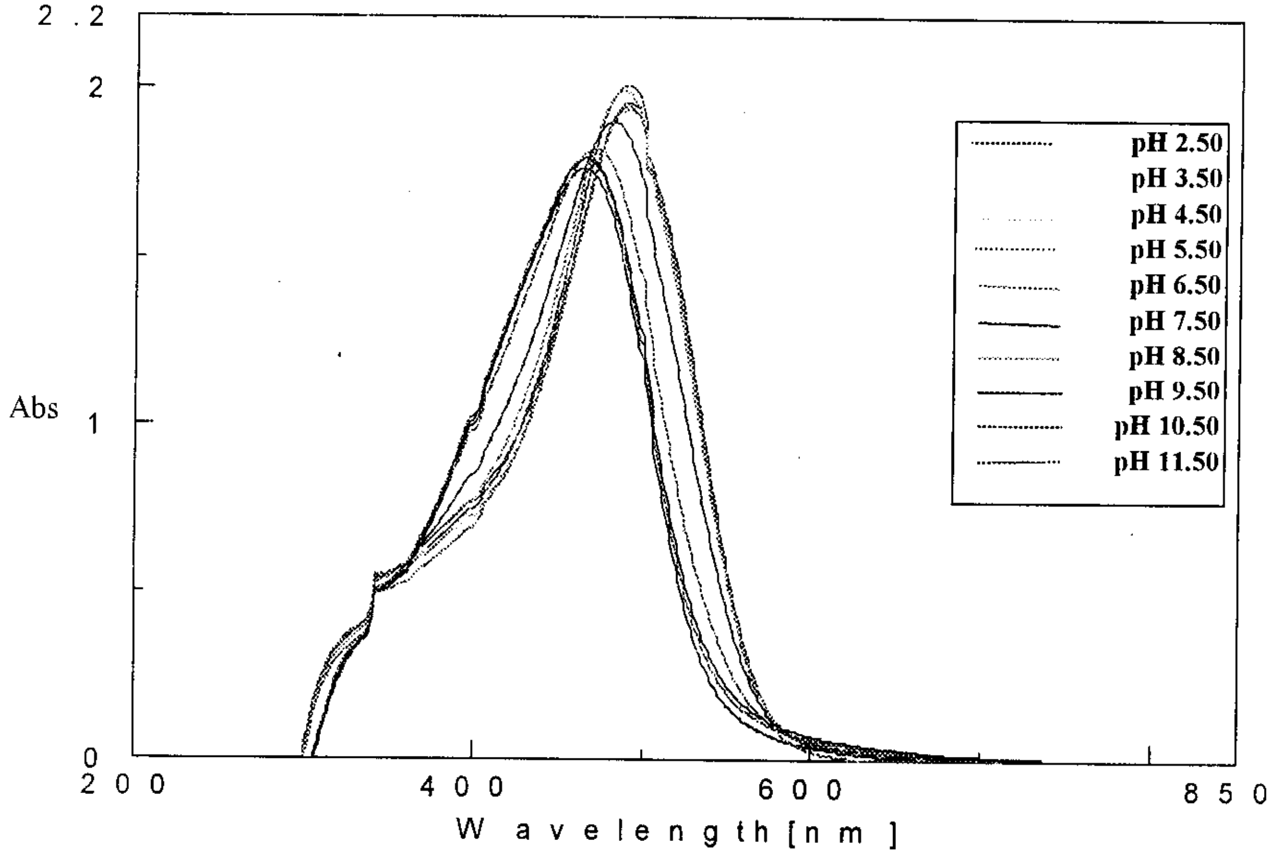
(a): Absorption spectra of (1.0 x 10^{-3} M) of reagent R_2 in buffer solutions of different pH values



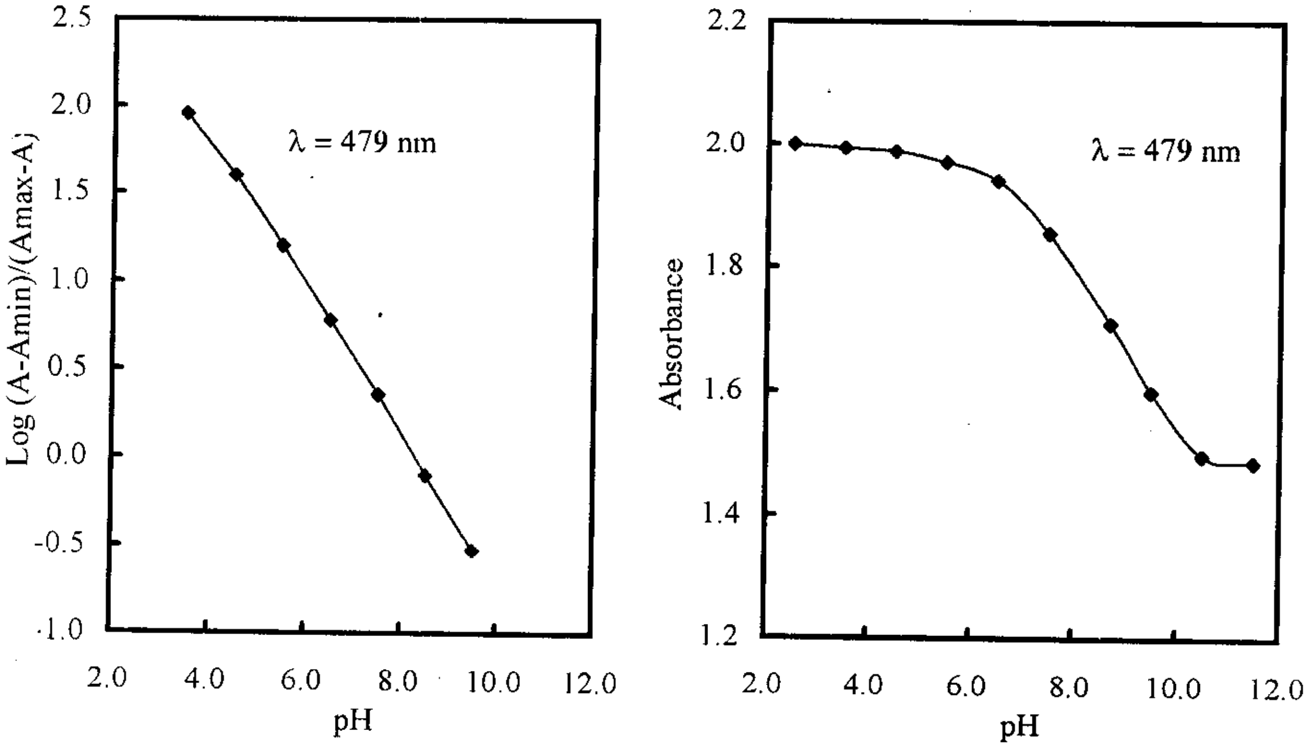
(c): The modified limiting method

(b): Half-height method

Fig. (7)



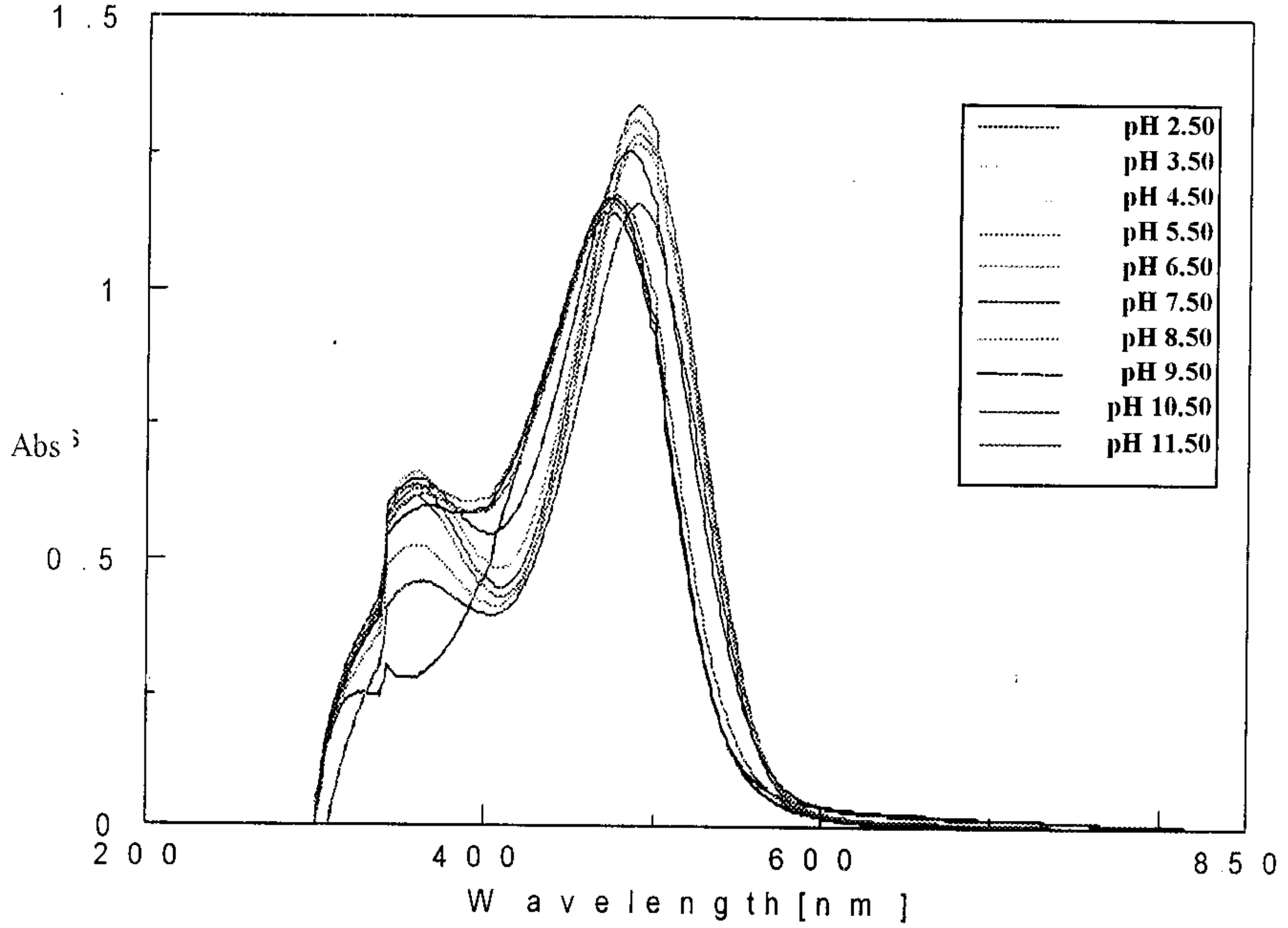
(a): Absorption spectra of (1.0×10^{-3} M) of reagent R_3 in buffer solutions of different pH values



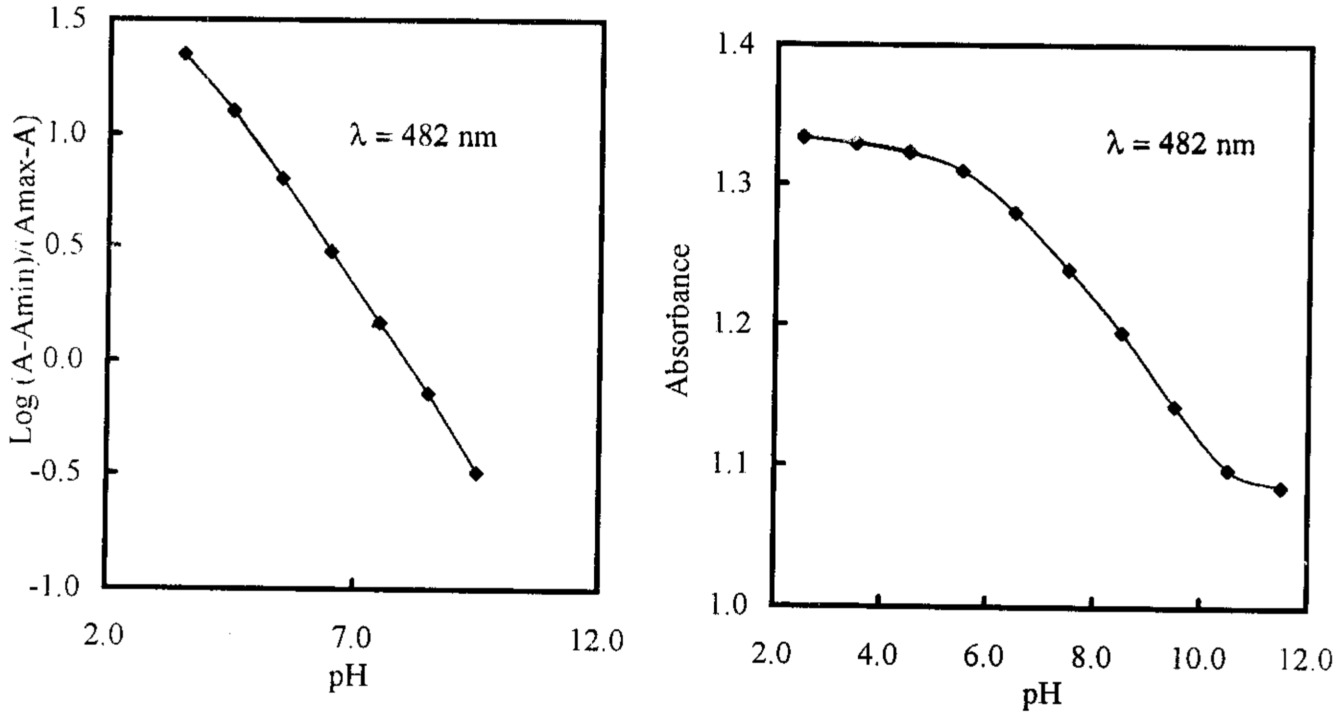
(c): The modified limiting method

(b): Half-height method

Fig. (8)



(a): Absorption spectra of (1.0 x 10^{-3} M) of reagent R_4 in buffer solutions of different pH values



(c): The modified limiting method

(b): Half-height method

Fig. (9)

Table (4): The ionization constsnts (pK_H) for azo dys reagents (R_1-R_8)

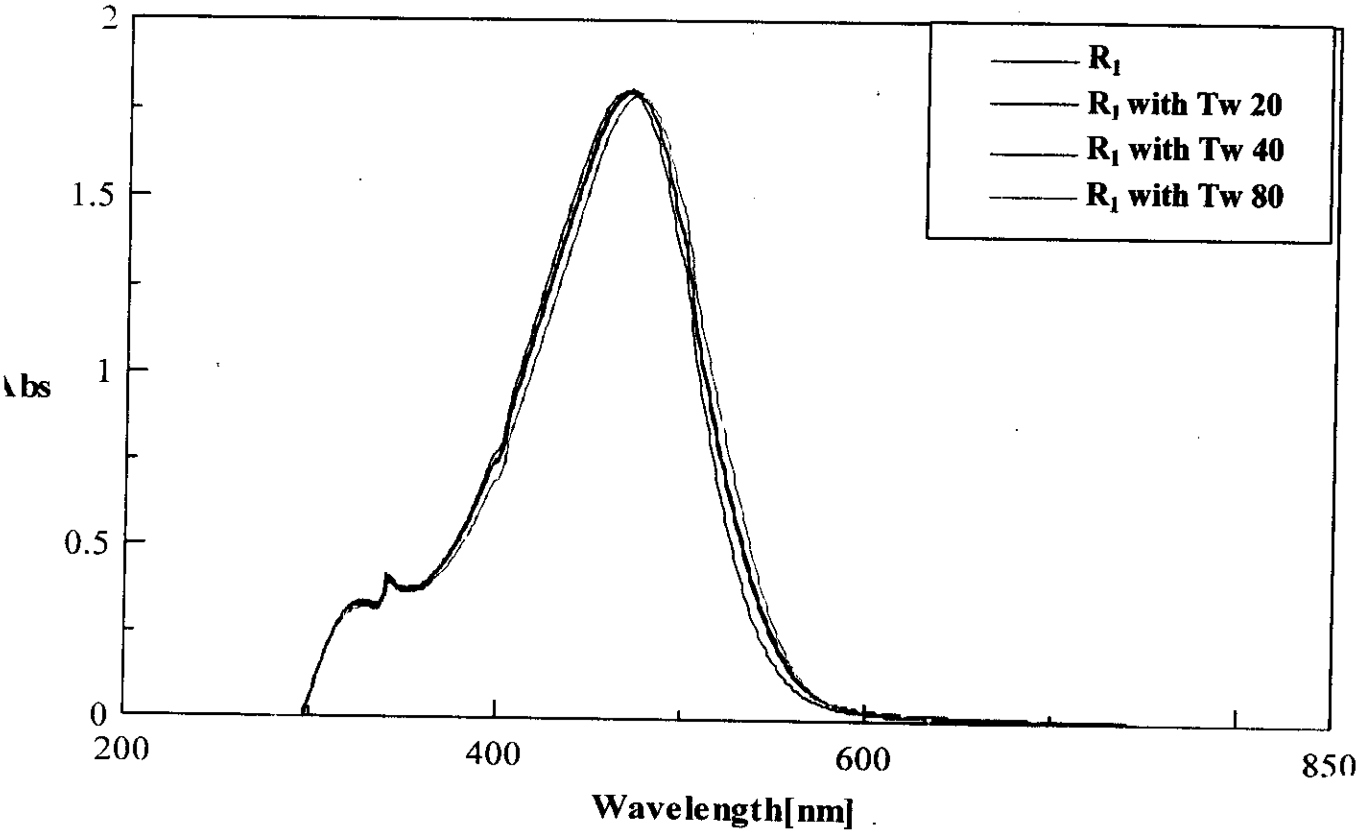
Reagent	Ionization constant	Method		Average
		a	b	
R_1	pK_H	7.89	8.21	8.05
R_2	pK_H	7.65	7.98	7.81
R_3	pK_H	7.75	8.31	8.03
R_4	pK_H	8.12	8.41	8.26
R_5	pK_H	7.91	8.12	8.01
R_6	pK_H	8.07	8.38	8.22
R_7	pK_{H1}	8.19	8.08	8.14
	pK_{H2}	8.34	8.51	8.43
R_8	pK_H	7.64	7.92	7.78

(a) The half-height method

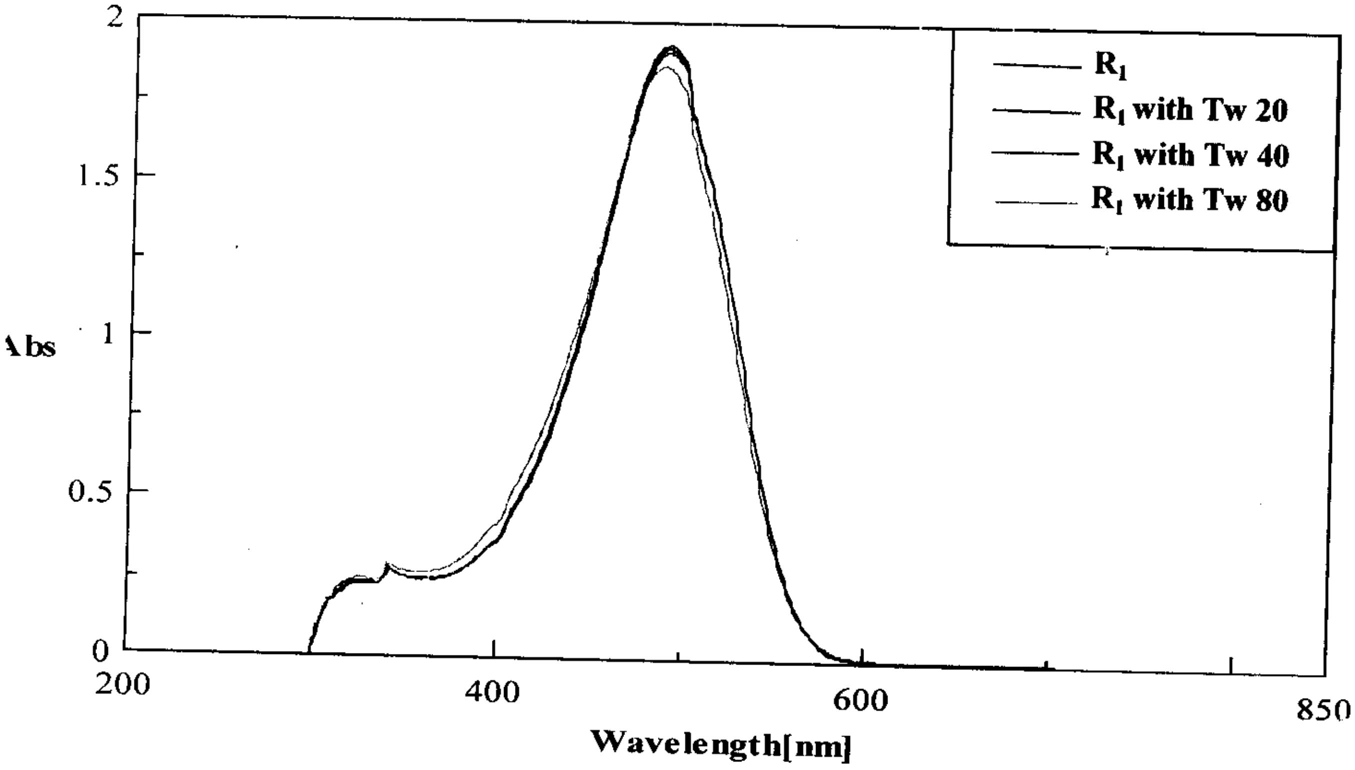
(b) The modified limiting method

3.4. The electronic spectra of reagents in the presence of surfactants

The effect of surfactants on the electronic absorption spectra of different reagents (***R_1-R_8***) is investigated in universal buffer solution. 0.5 ml of 2.0×10^{-3} M of reagent , 2.0 ml of buffer solution and 1.0 ml of 0.5% (v/v) of surfactnt are mixed well and the volume is completed to 10 ml with bidistilled water. The absorbance is measured against a blank solution containing buffer and surfactant only. Representation curves in case of ***R_1*** at pH 5.50 and 9.50 are shown in Fig. (10). The results show that the addition of surfactants hav no any effect on the band of reagent. Tha same results are obtained with the other reagents.



(a): Effect of different surfactants on the spectra of (1.0 x 10^{-3} M) of reagent R_1 at pH 9.50



(b): Effect of different surfactants on the spectra of (1.0 x 10^{-3} M) of reagent R_1 at pH 5.50

Fig. (10)

3.5. Spectrophotometric studies of metal complexes in solution

The importance of metal complexes comes from the idea that the complex required new properties may be strikingly different from those of the original metal ion. Such changes include those in colour, stability toward oxidation or reduction, magnitude of ionic charge and solubility. These new properties enable us to identify and determine the metal ion or the coordinated agent illustrate applications of complexing agent in analytical chemistry..

The following points are investigated for studying the metal complexes of Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^{+} ions with azo dyes under consideration

1- Effect of pH
2- Determination of λ_{max}
3- Effect of surfactants
4- Effect of reagent concentration
5- Effect of time and temperature
6- Effect of sequence of addition
7- Mole ratio of the complexes
8- Stability constants of the complexes
9- Spectrophotometric determination of metal ions
    a) Obeyence of ***Beer's*** law
    b) ***Ringbom*** method
10-Interferences
11-Analytical applications

3.5.1. Spectrophotometric studies of [2-amino-4-(phenylazo)pyridine-3-ol] R_1, [2-amino-4-(3-chlorophenylazo)pyridine-3-ol] R_2 complexes with Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^+ ions

In order to investigate the optimum conditions for metal-reagent complexes, the following studies of different variables should be taken into consideration as recorded below.

3.5.1.1. Effect of pH

The effect of pH on the complex formation between the metal ions under investigation and reagents R_1 and R_2 is studied in universal buffer solution of pH values (2.5-11.5). A solution of (1.0 ml of 2.0×10^{-3} M) reagent R_1 or R_2 in DMF, (1.0 ml of 1.0×10^{-3} M) metal ions and 2.0 ml buffer of different pH values are mixed well. The volume of this mixture is completed to 10 ml by bidistilled water. The absorption spectra are recorded using a blank solution prepared by the same manner without metal ions of the same pH value.

The careful investigation of the spectra shows that the optimum pH value recommended for subsequent studies of metal-reagent complexes with Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^+ are as recorded in Tables (5 and 6) on using reagents R_1 and R_2, respectively. Furthermore, The amount of buffer is examined by adding different volumes (1.0, 2.0,...,6.0 ml) of selected pH to both sample and blank. The experiments show that 2.0 ml from the selected pH are sufficient to produce high absorbance for all metal complexes with R_1 and R_2 as shown in Fig's. (11 and 12).

3.5.1.2. Determination of maximum wavelength of complex species

The value of the λ_{max} at which each complex species is absorbed is investigated in universal buffer midium. A representation curves (A-D) in case of Ag-R_1 and Cu-R_2 complexes are shown in Fig. (13). The investigation of these cureves shows that, reagent R_1 is absorbed maximally at 467 nm at pH 9.50 with buffer as a blank (curve A), whereas the complex of Ag^+ shows absorption band at 482 nm with buffer as a blank (curve B). Using reagent R_1 and buffer as a blank (curve C), the complex of Ag^+ is absorbed at 524 nm. The addition of (2.0 ml of 0.5% v/v) Tween 20 in the sample and blank (curve D) increased the absorbance and shift the λ_{max} of Ag^+ complex from 524 to 531 nm. The λ_{max} of Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} complexes with reagent R_1 are recorded in Table (5).

For reagent R_2 it is found that, the reagent is absorbed maximally at 482 nm in pH 5.50 with the buffer as a blank (curve A), while the complex of Cu^{2+} showed absorption band at 492 nm with buffer as a blank (curve B). Using reagent R_2 and buffer as a blank, the complex has maximum absorption at 555 nm, (curve C). The addition of (2.0 ml of 0.5% v/v)Tween 40 in both sample and blank (curve D) increases the absorbance and causes slighty shift in the λ_{max} of Cu^{2+} complex from 555 to 560 nm .

On the other hand, the experiments show that the addition of (2.0 ml of 0.5% v/v) Tween 20 for Ni^{2+} and Ag^+ complexes with reagent R_2 increases the absorbance and shifts the λ_{max} from 540 and 531 to 550 and 543 nm, respectively, while the addition of (2.0 ml of 0.5% v/v) Tween 40 increases the absorbance and causes silight shift in the λ_{max} of Hg^{2+} complex. Whereas Co^{2+} complex shows no change neither in absorbance nor in the λ_{max} as recorded in Table (6).

3.5.1.3. Effect of surfactants

Different surfactants as Tween 20, 40, 80, Triton X-100, sodium dodecyl sulfate (SDS) and cetylpyridnium chloride (CPS) are used to improve the formation of metal complexes. (0.5, 1.0,...., 3.0 ml of 0.5% v/v or w/v) from each surfactant is added to sample containing (1.0 ml of 1.0 x10^{-3} M) metal ion, 2.0 ml of the selected pH and (1.0 ml of 2.0 x 10^{-3} M) R_1 or R_2. The volume is completed to the mark in 10 ml measuring flask by bidistilled water. The absorbance is measured against a blank solution containing the same volume of surfactant.

The results obtained show that (2.0 ml of 0.5% v/v) Tween 20 is sufficient to increase the absorbance in case of Ag^+ with reagent R_1 and shifts the λ_{max} by 7.0 nm while the complexes of Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} ions show no response for any of the above surfactants.

On using reagent R_2, it is found that (2.0 ml of 0.5% v/v) Tween 20 increases the absorbance of Ni^{2+} and Ag^+ and shifts their λ_{max} by 10 and 12 nm respectively. On the other hand (2.0 ml of 0.5% v/v) Tween 40 is sufficient to increase the absorbance of Cu^{2+} and Hg^{2+} complexes and causes shift in the λ_{max} by 5.0 nm. Co^{2+} complex shows no changes in the absorbance or in the position of maximum wavelength. The role of addition of surfactants is that to increase the solvation of the formed complexes and hence increase the deepenss of the coloure that leades to increase in the absorbance value and slighty shfit in the wavelength of the formed complexes. Also the addition of surfactants enhancing the sensitivity, selectivity and molar absorptivity during the determination of metal ions.

3.5.1.4. Effect of reagent concentration

To study the effect of reagents ***R_1*** and ***R_2*** concentration on the complex formation, the concentration of metal ions is kept constant at (1.0 ml of 1.0 x 10^{-3} M), while that of the reagent is regularly varied (0.2, 0.5,...., 2.5 ml of 2.0 x 10^{-3} M) and 2.0 ml of the selected pH is added in 10 ml calibrated flask. The volume is completed with bidistilled water to the mark. For metal that shows a sensitivity for the surfactant, optimum omount of surfactant is added in both sample and blank. The absorption spectra are recorded against reagent blank solution prepared in the same manner without metal ion. The absorbance is then plotted against ml added of reagent as shown in Fig's. (14 and 15). The optimum reagents concentration is recorded in Tables (5 and 6).

3.5.1.5. Effect of time and temperature

The effect of time on metal-reagent complexes is studied by measuring the absorbance of a solution containing the metal ion and the reagent in optimum buffer and surfactant against blank solution prepared by the same way without metal ions in the visible region at various time intervals.

Also the effect of temperature is studied for the same sample by heating the sample solution and the blank at different temperatures (25-70 °C). After cooling the sample and blank to room temperature, the absorbance is measured at the recommended λ_{max}.

Experiments show that the complexes are formed instantaneously after mixing the metal and the reagent and remain constant for 24 h. Also it is found that no change in the absorbance occurred on increasing the temperature up to 60 °C above which the absorbance begins to fade slowly.

3.5.1.6. Effect of sequence of additions

The effect of sequence of additions on the complex formation is investigated by measuring the absorbance of solutions prepared by different sequence of addition in the visible region against a blank solution prepared in the same manner. Experiments show that the order (reagent-buffer-surfactant-metal) givea the best results for Ag^+ complex with $\boldsymbol{R_1}$ whil the order (reagent-buffer-metal) is favoured for Co^{2+}, Ni^{2+}, Cu^{2+} and Hg^{2+} complexes with tha same reagent. Using $\boldsymbol{R_2}$, the order (reagent-buffer-surfactant-metal) is favoured for Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^+ complexes, while the order (reagent-buffer-metal) is favoured for Co^{2+} complex. Other sequences give lower absorbance values under the same conditions.

3.5.1.7. Molecular structure of metal complexes

Investigation of the molecular composition of complexes of Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^+ ions with reagents $\boldsymbol{R_1}$ and $\boldsymbol{R_2}$ is made by using the following methods

a) Molar ratio method

The molar ratio method described by ***Yoe*** and ***Jones***(111) is used to study the stoichiometry of the complexes between metal ions Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^+ and the reagents $\boldsymbol{R_1}$ and $\boldsymbol{R_2}$. In this method, variable concentrations of reagent $\boldsymbol{R_1}$ or $\boldsymbol{R_2}$ (0.2-2.4 ml of 1.0×10^{-3} M) are mixed with (0.5 ml of 1.0×10^{-3} M) of metal ions and 2.0 ml of the selected pH then the volume is completed to 10 ml with bidistilled water.

For metals that have sensitivity for the surfactant, the above method is repeated with the addition of (2.0 ml of 0.5% v/v) Tween 20 for Ag^+ with $\boldsymbol{R_1}$, Tween 20 for Ni^{2+} and Ag^+ with $\boldsymbol{R_2}$, Tween 40 for Cu^{2+} and Hg^{2+} with $\boldsymbol{R_2}$. The absorbance is measured at the recommended λ_{max} against a blank solution prepared by the same way without metal ion. The absorbance values are then plotted against the molar ratio [R/M] as shown in Fig's. (16-19).

Experimental results show that the complexes of Co^{2+}, Ni^{2+} and Ag^{+} with R_1 are formed as 2:1 (R:M) ratio while the complexes of Cu^{2+} and Hg^{2+} are formed as 1:1 (R:M) with the same reagent. On using R_2, all metal complexes are formed as 2:1 (R:M).

b) Continuous variation method

The modification of ***Job's*** continuous variation method^(112)^ performed by ***Vesbourgh et al***^(113)^ is utilized for investigating the reaction between the metal ions and the reagent. Different volumes (0.2, 0.4,..., 1.8 ml of 1.0 x 10^{-3} M) of metal and reagent are mixed well while keeping the total molar concentration constant at (2.0 x 10^{-4} M). 2.0 ml of the selected pH is added and the volume is completed to the mark by bidistilled water. For the metals that have sensitivity for the surfactant the above method is repeated with addition of the optimum amount of surfactant in the sample and blank as in the molar ratio method.

The absorbance for each sample solution is measured against blank solution. The absorbance is then plotted against the mole fraction of the reagent as shown in Fig's. (16-19). The results obtained by this method are in agreement with that obtained by the molar ratio method.

3.5.1.8. Stability constant of the formed complexes

Spectrophotometric methods can be applied for the determination of the stability constant of the complexes. Generally the spectrophotometric methods that are usually applied to the stoichiometric ratio of the complexes can be also used for the determination of their stability constants in solution. The overall formation constants of the concerned complexes are calculated using the data of the molar ratio and continuous variation methods applying ***Issa***(114) method. In this method the spectrophotometric data of the molar ratio and continuous variation methods utilized for studying the stoichiometry of the complexes are substituted in the following equation:

$$K_n = \frac{A/A_m}{(1-A/A_m)^{n+1}\, C_R^{\,n}\, n^2}$$

Where:

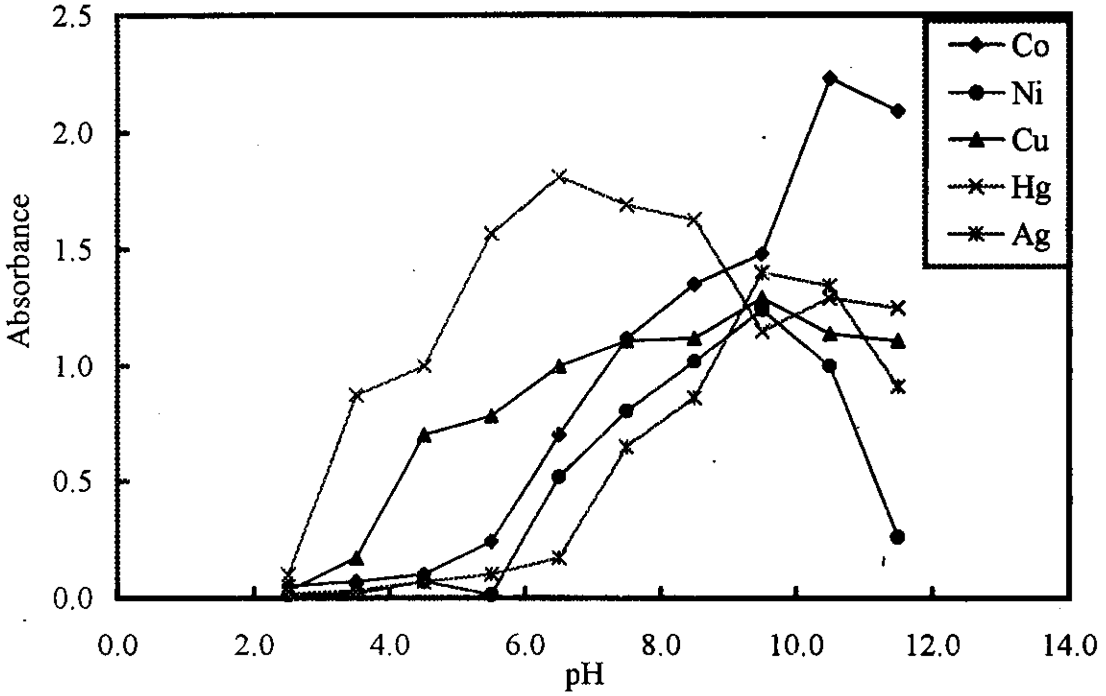
A, is the absorbance at reagent concentration C_R

A_m, is the maximum absorbance of the complex

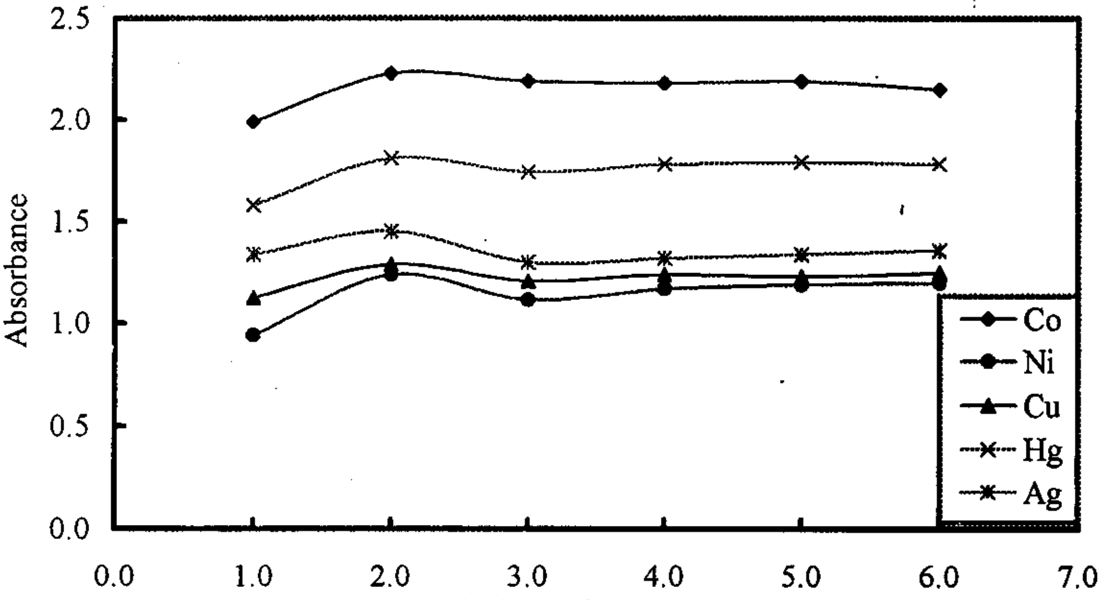
n, is the stoichiometric ratio of the complex

K_n, is the stability constant

The apparent stability constant of the different metal complexes with the reagents R_1 and R_2 are recorded in Tables (5 and 6). The careful investigation of the results obtained indicates that the metal complexes have high stability constant values. Also the stabiliy constant of some metal ions incraes with the addition of surfactant like Ag^+ complex with reagent R_1 showed higher stability constant value with the addition of (2.0 ml of 0.5% v/v)Tween 20. On using reagent R_2, the addition of (2.0 ml of 0.5% v/v) Tween 20 in case of Ni^{2+} and Ag^+ increase their stability constant while the addition of (2.0 ml of 0.5% v/v) Tween 40 in case of Cu^{2+} and Hg^{2+} with the same reagent leads to higher stability constant values in comparison with the same complexes without the addition of surfactant.

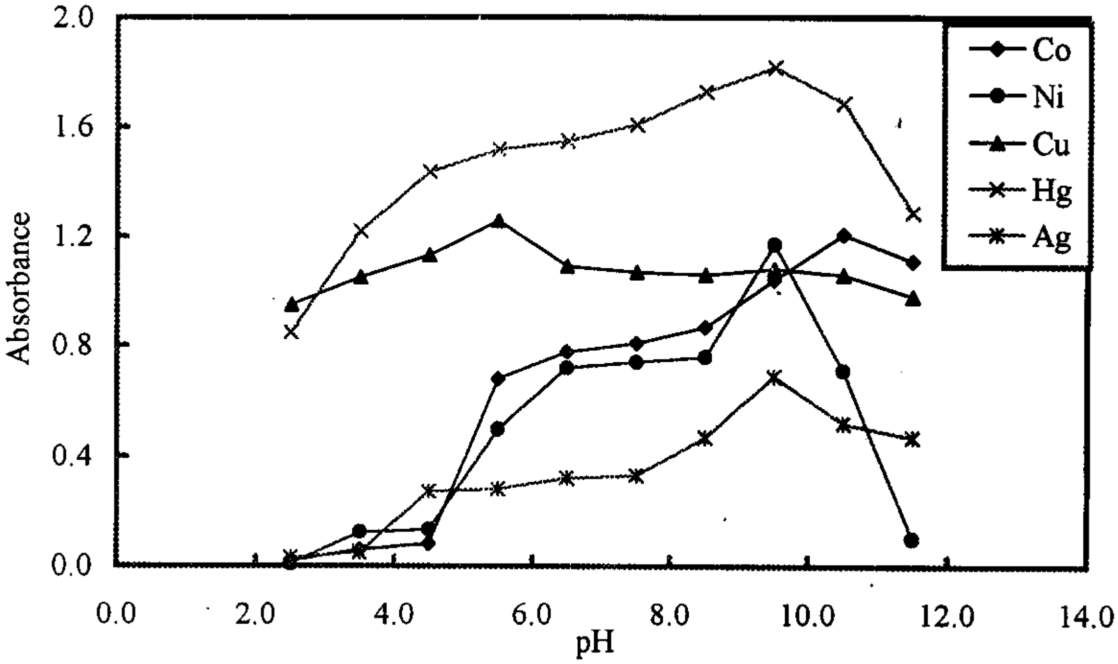


(a): Effect of pH on the maximum absorbances of metal ions complexes using (2.0×10^{-3} M) of reagent R_1

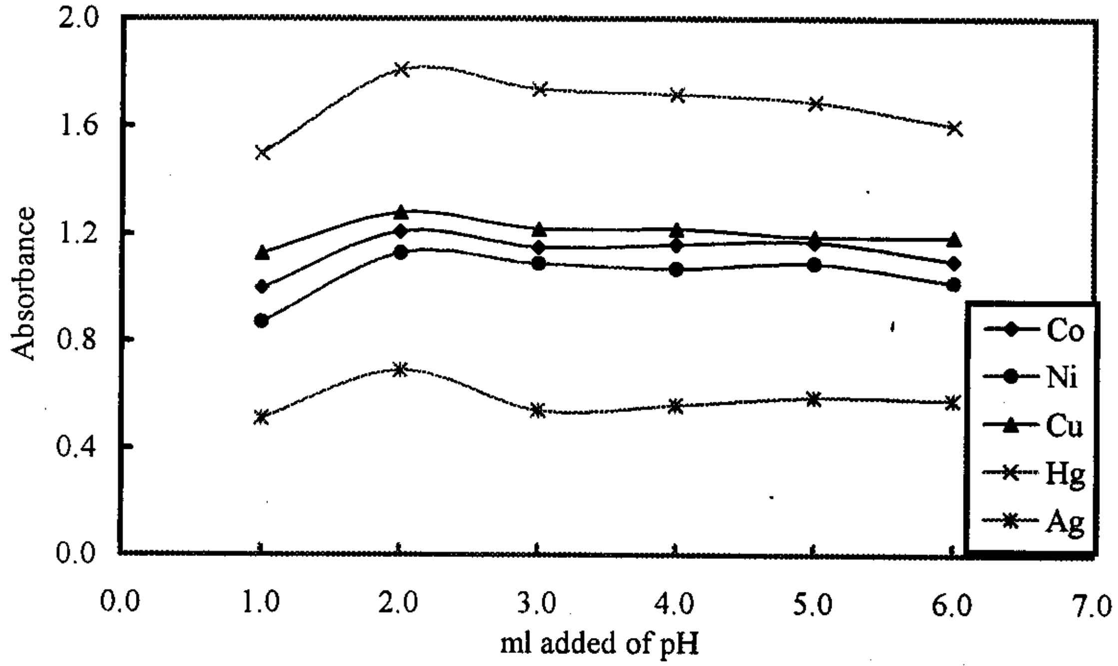


(b): Effect of ml added of buffer solution of the recommended pH on the complex formation using (2.0×10^{-3} M) of reagent R_1

Fig. (11)

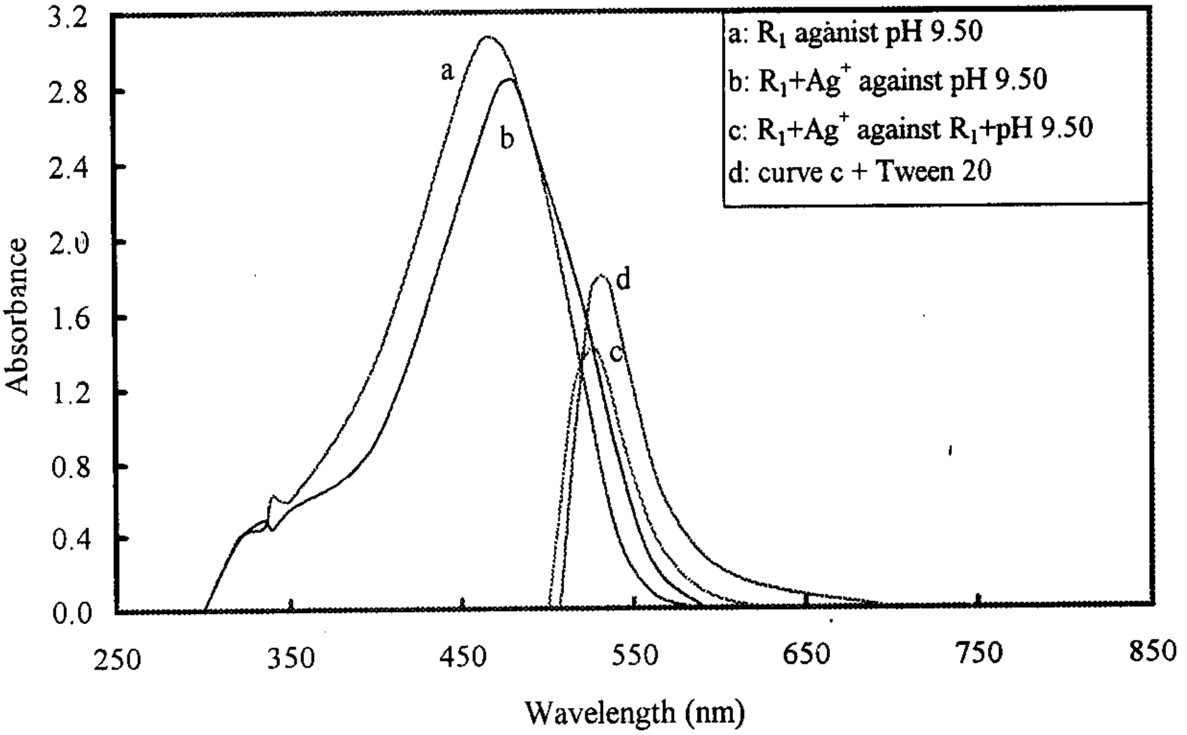


(a): Effect of pH on the maximum absorbances of metal ions complexes using (2.0 x 10^{-3} M) of reagent R_2

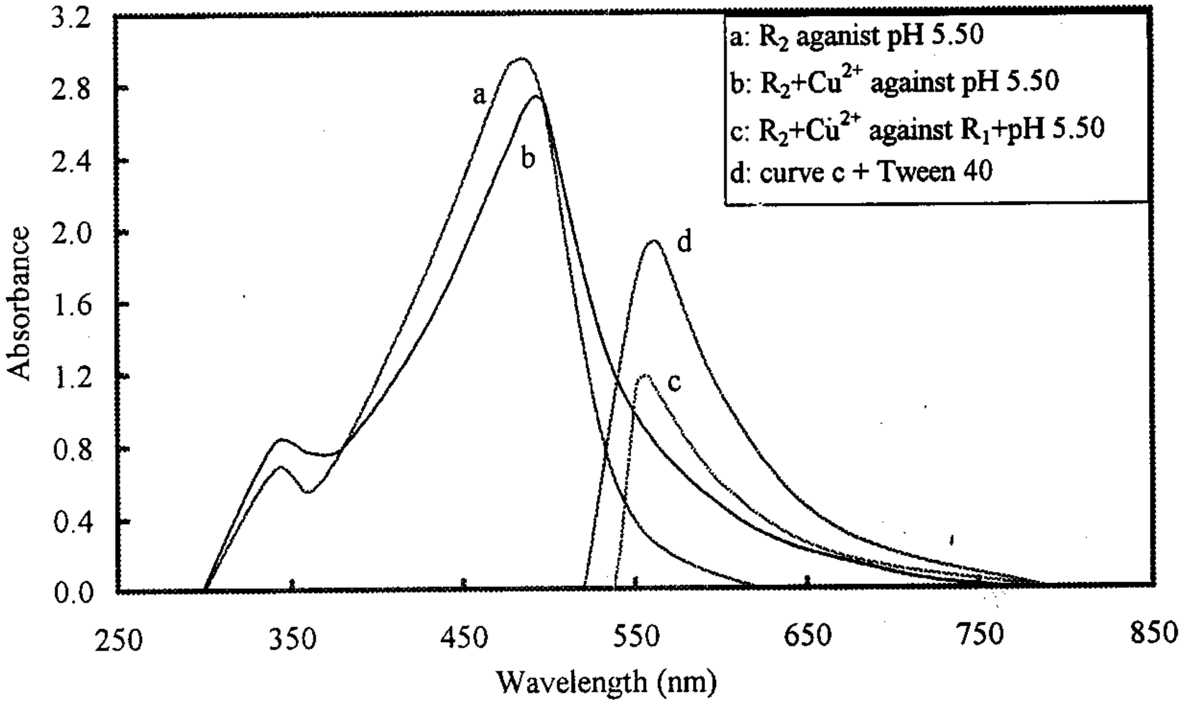


(b): Effect of ml added of buffer solution of the recommended pH on the complex formation using (2.0 x 10^{-3} M) of reagent R_2

Fig. (12)

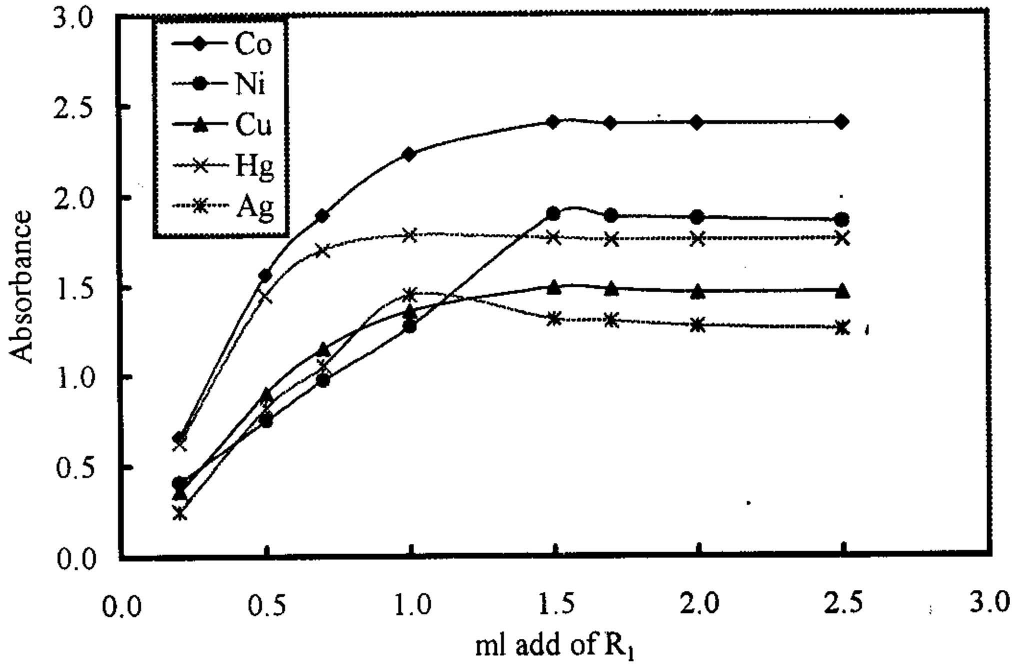


(a): Absorption spectra of (2 .0 x 10^{-3} M) R_1 with (1.0 x 10^{-3} M) of Ag^+ in the presence of (2.0 ml 0.5% v/v) Tween 20

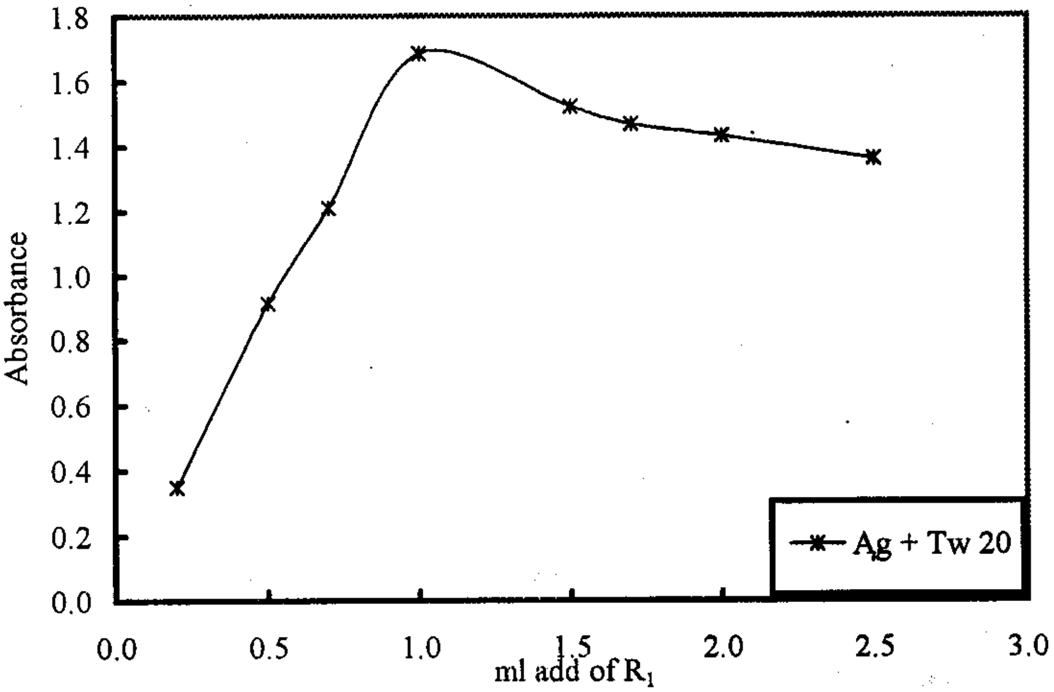


(b): Absorption spectra of (2 .0 x 10^{-3} M) R_2 with (1.0 x 10^{-3} M) of Cu^{2+} in the presence of (2.0 ml 0.5% v/v) Tween 40

Fig. (13)

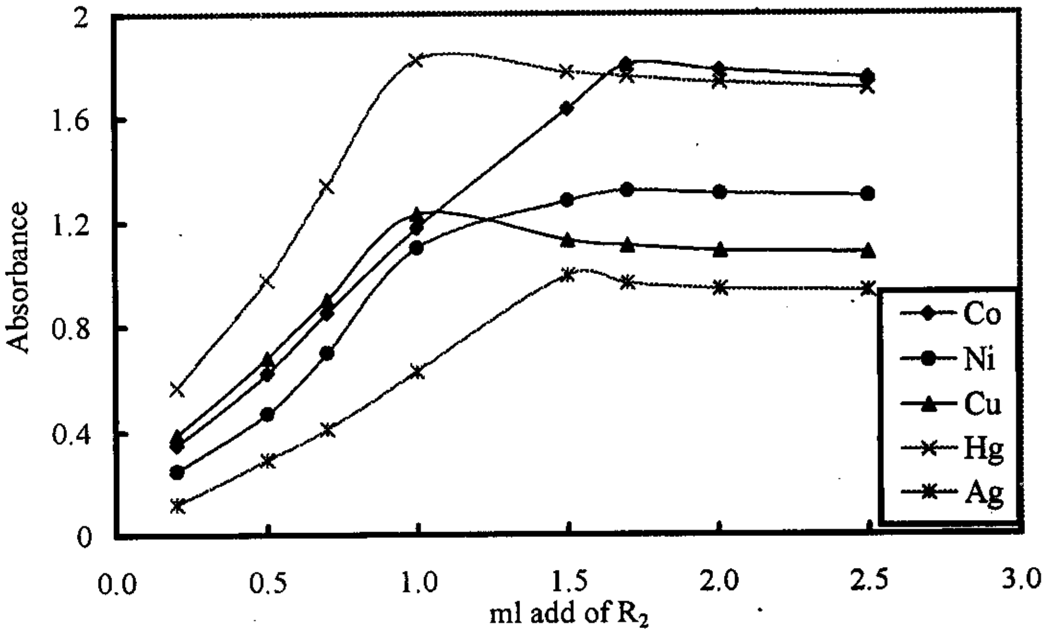


(a): Effect of reagent concentration on the absorbance of metal ions complexes using (2.0×10^{-3} M) of reagent R_1

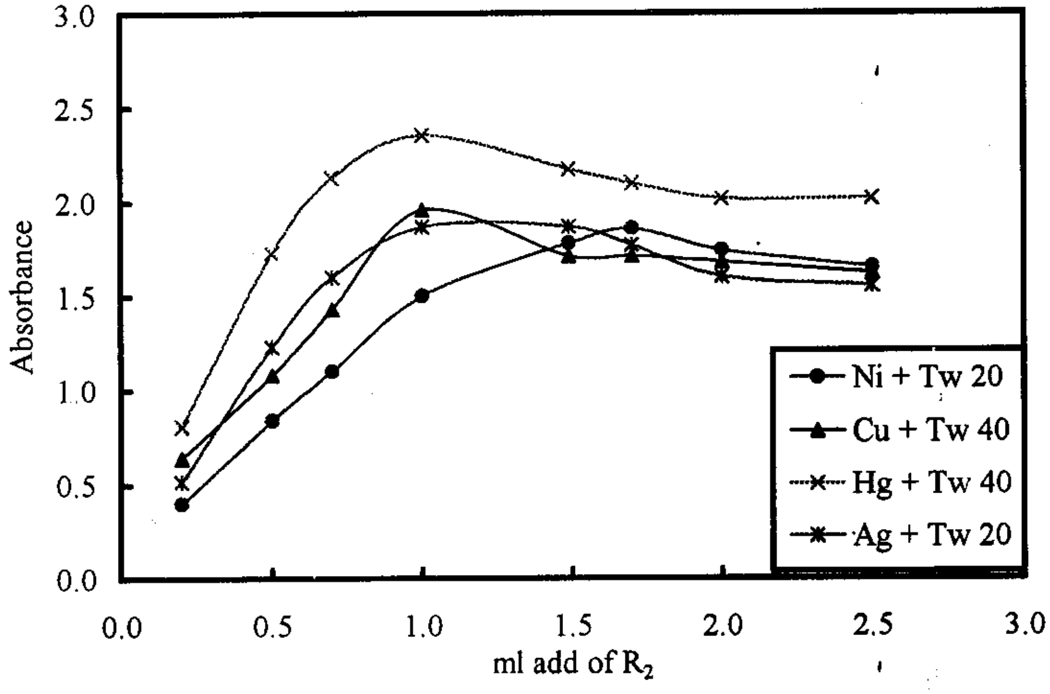


(b): Effect of reagent concentration on the absorbance of metal ions complexes in the presence of surfactant using (2.0×10^{-3} M) of reagent R_1

Fig. (14)

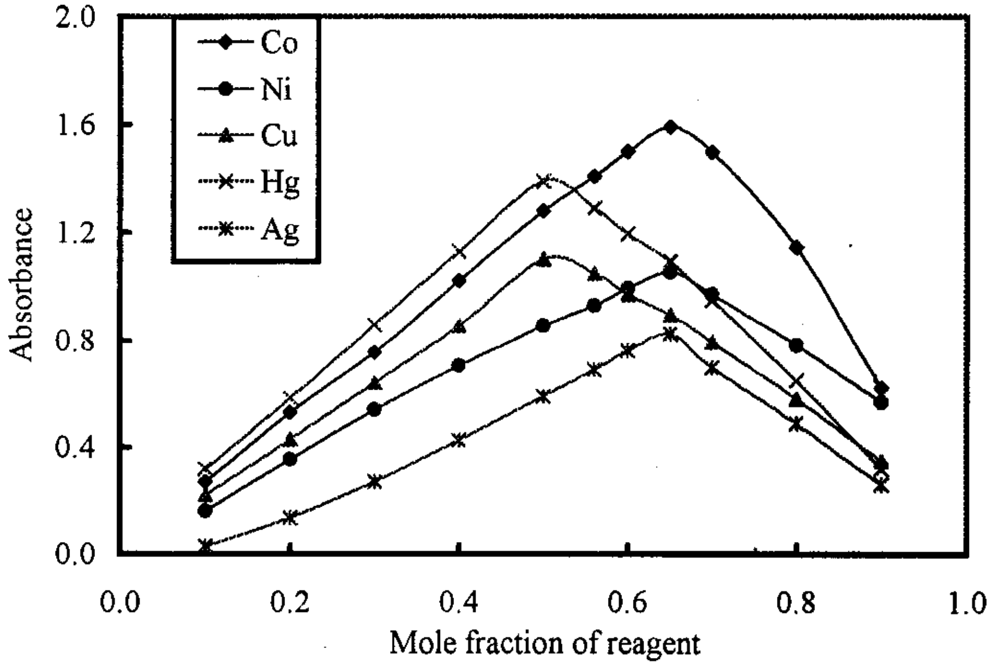


(a): Effect of reagent concentration on the absorbance of metal ions complexes using (2.0 x 10^{-3} M) of reagent R_2

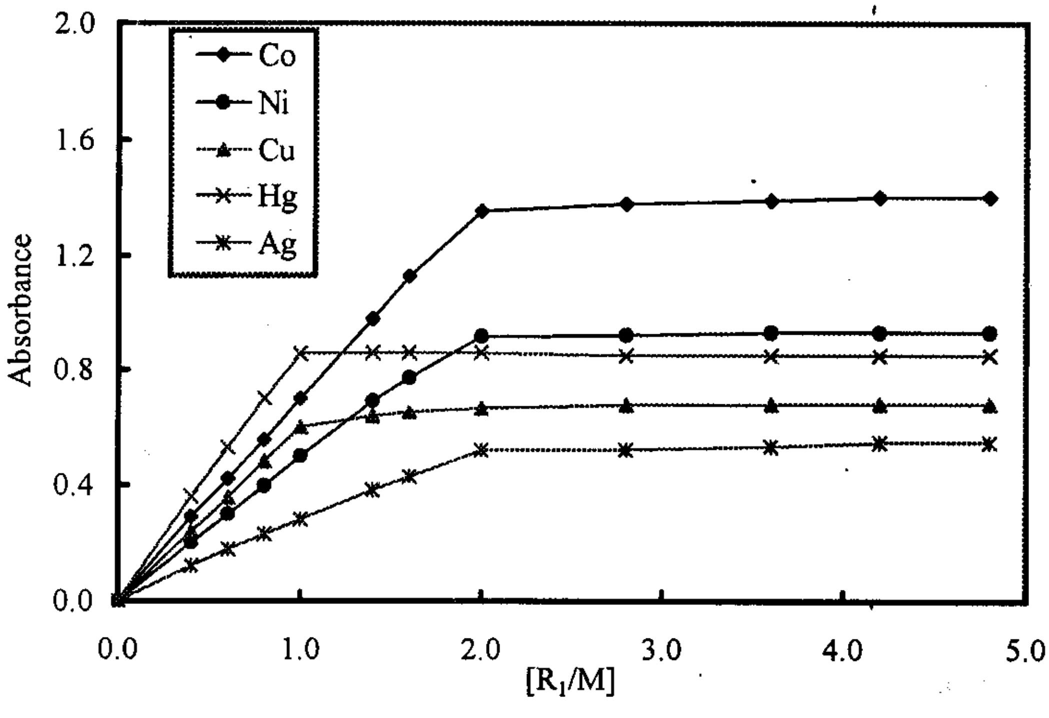


(b): Effect of reagent concentration on the absorbance of metal ions complexes in the presence of surfactants using (2.0 x 10^{-3} M) of reagent R_2

Fig. (15)

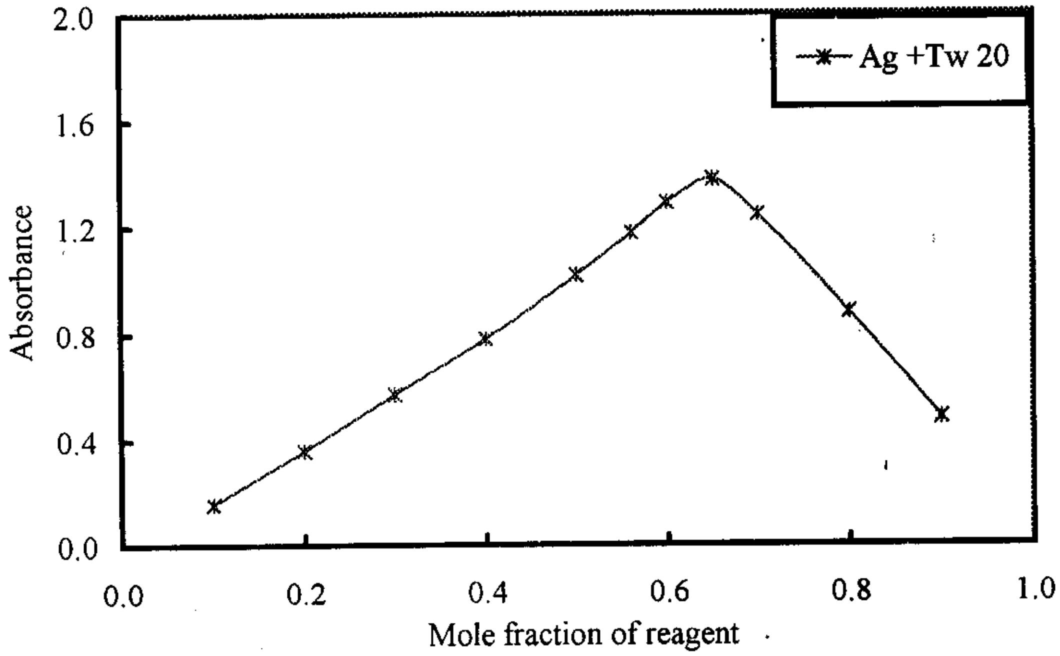


(a): Continuous variation using (1.0 x 10^{-3} M) of reagent R_1 with (1.0 x 10^{-3} M) of metal ions

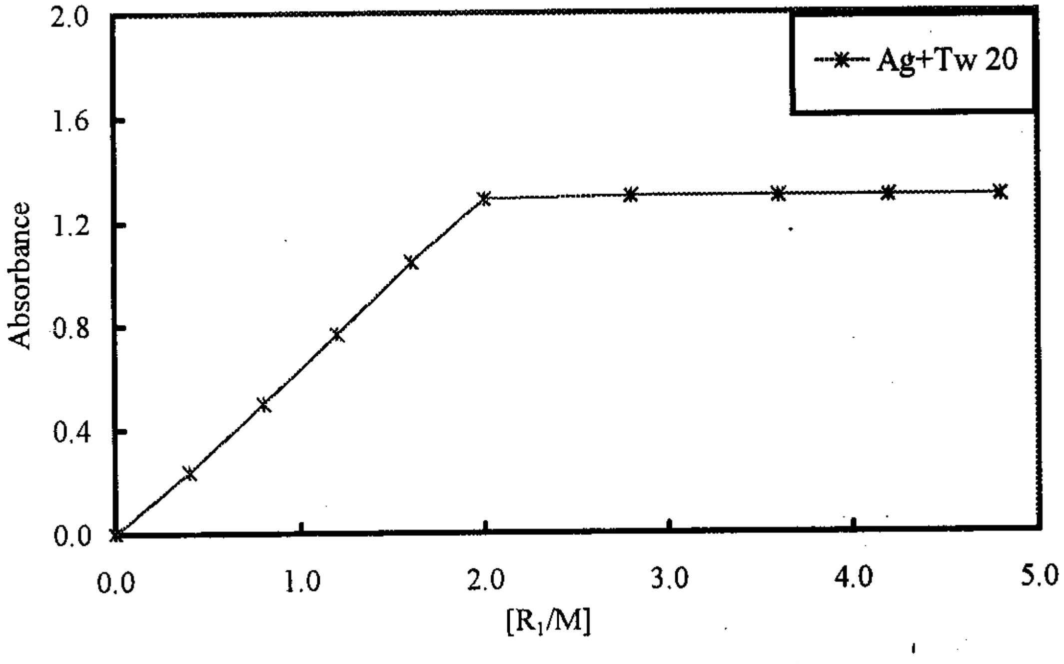


(b): Molar ratio using different volumes of (1.0 x 10^{-3} M) of reagent R_1 with (0.5 ml of 1.0 x 10^{-3} M) of metal ions

Fig. (16)

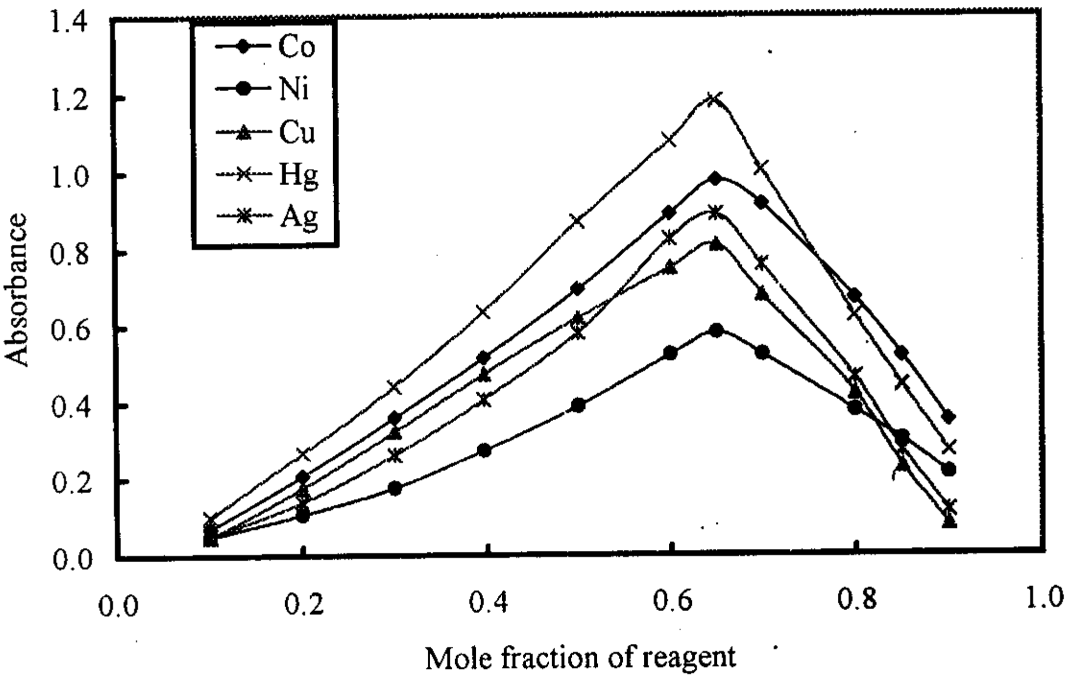


(a): Continuous variation using (1.0 x 10^{-3} M) of reagent R_1 with (1.0 x 10^{-3} M) of metal ion in the presence of surfactant

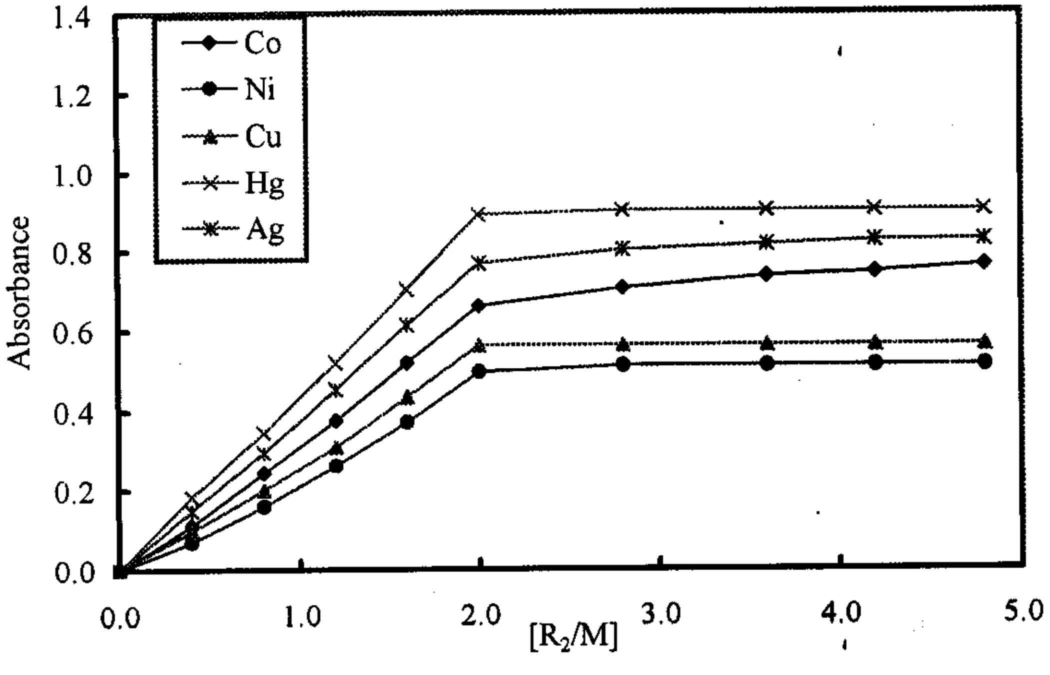


(b): Molar ratio using different volumes of (1.0 x 10^{-3} M) of reagent R_1 with (0.5 ml of 1.0 x 10^{-3} M) of metal ion in the presence of surfactant

Fig. (17)

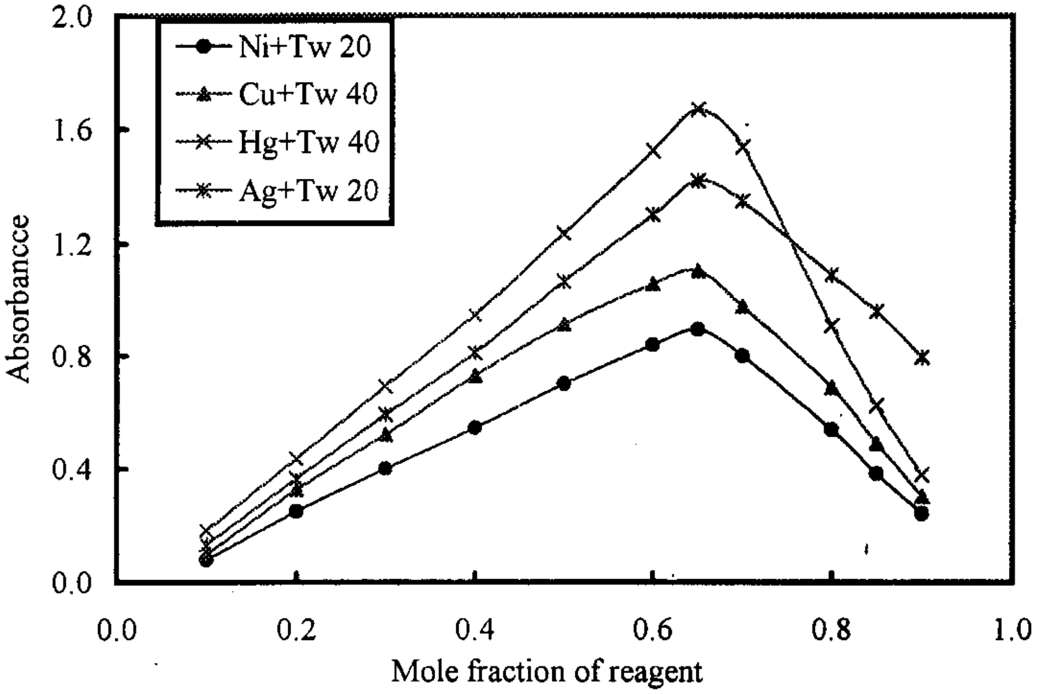


(a): Continuous variation using (1.0 x 10^{-3} M) of reagent R_2 with (1.0 x 10^{-3} M) of metal ions

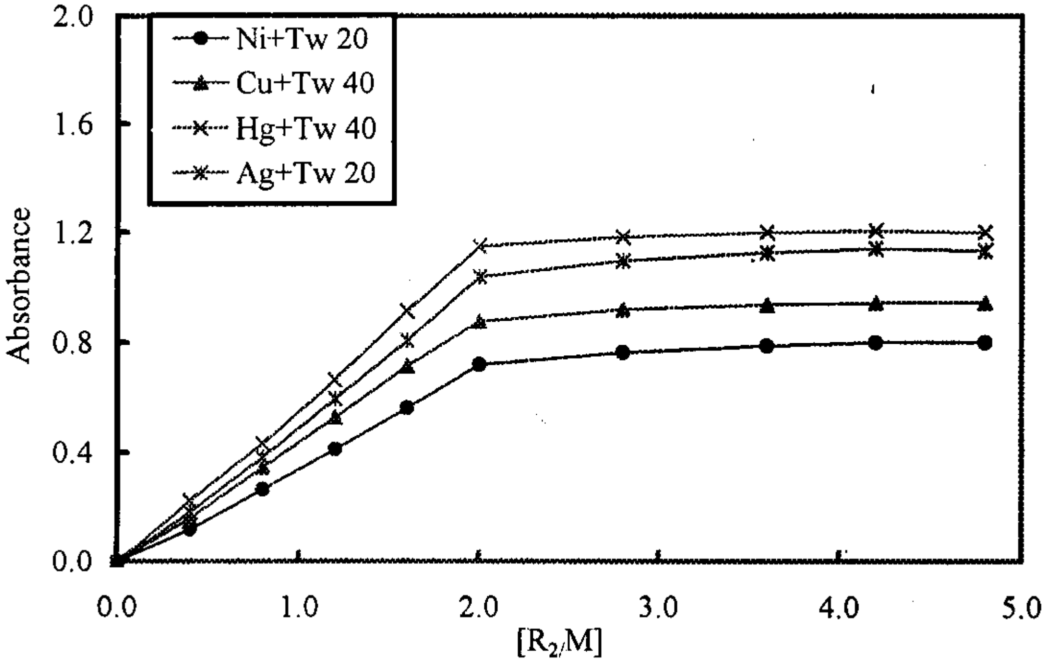


(b): Molar ratio using different volumes of (1.0 x 10^{-3} M) of reagent R_2 with (0.5 ml of 1.0 x 10^{-3} M) of metal ions

Fig. (18)



(a): Continuous variation using (1.0×10^{-3} M) of reagent R_2 with (1.0×10^{-3} M) of metal ions in the presence of surfactants



(b): Molar ratio using different volumes of (1.0×10^{-3} M) of reagent R_2 with (0.5 ml of 1.0×10^{-3} M) of metal ions in the presence of surfactants

Fig. (19)

Table (5): Optimum condition parameters of reagent R_1 with the ions Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^{+}

Parameters	R_1					
	Co^{2+}	Ni^{2+}	Cu^{2+}	Hg^{2+}	Ag^{+}	
					Without^S	With^S
Working pH	10.50	9.50	9.50	6.50	9.50	9.50
λ_{max} **(nm)**	540	534	542	552	524	531
Buffer volume	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
Reagent concentration (2.0×10^{-3} M)	1.5 ml	1.5 ml	1.5 ml	1.0 ml	1.0 ml	1.0 ml
Surfactant type	---	---	---	---	---	Tween 20
Surfactant volume	---	---	---	---	---	2.0 ml
Sequence of addition	R-B-M	R-B-M	R-B-M	R-B-M	R-B-M	R-B-S-M
Stoichiometric ratio (R:M)	2:1	2:1	1:1	1:1	2:1	2:1
Stability constant[a]	10.06	11.54	7.15	6.84	8.85	12.99
Stability constant[b]	10.72	11.97	7.59	6.25	8.35	12.49
Average of stability constant	10.39	11.76	7.37	6.55	8.60	12.74

R: reagent B: Buffer ^{S}: surfactant M: metal

[a]: Stability constant using molar ratio method

[b]: Stability constant using contanous variation method

Table (6): Optimum condition parameters of reagent R_2 with the ions Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^{+}.

Parameters	**R_2**								
	Co^{2+}	**Ni^{2+}**		**Cu^{2+}**		**Hg^{2+}**		**Ag^{+}**	
		Without^{S}	**With^{S}**	**Without^{S}**	**With^{S}**	**Without^{S}**	**With^{S}**	**Without^{S}**	**With^{S}**
Working pH	10.50	9.50	9.50	5.50	5.50	9.50	9.50	9.50	9.50
λ_{max} (nm)	547	540	550	555	560	560	565	531	543
Buffer volume	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
Reagent concentration (2.0×10^{-3} M)	1.7 ml	1.7 ml	1.7 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.5 ml	1.0 ml
Surfactant type	---	---	Tween 20	---	Tween 40	---	Tween 40	---	Tween 20
Surfactant volume	---	---	2.0 ml	---	2.0 ml	---	2.0 ml	---	2.0 ml
Sequence of addition	R-B-M	R-B-M	R-B-S-M	R-B-M	R-B-S-M	R-B-M	R-B-S-M	R-B-M	R-B-M
Stoichiometric ratio (L:M)	2:1	2:1	2:1	2:1	2:1	2:1	2:1	2:1	2:1
Stability constant^{a}	10.90	9.46	11.97	10.23	12.43	9.53	11.40	9.92	13.83
Stability constant^{b}	10.78	9.91	11.63	10.31	12.24	9.76	11.23	10.20	13.45
Average of stability constant	10.84	9.69	11.80	10.27	12.34	9.65	11.32	10.06	13.64

R: reagent B: Buffer ^{S}: surfactant M: metal

^{a}: Stability constant from molar ratio method

^{b}: Stability constant from contanous variation method

3.5.1.9. Spectrophotometric determination of metal ions

a) Validity of Beer's law

Under optimum conditions of pH, surfactants, reagent concentration, sequence of addition, time and temperature, metals are allowed to react with azo dye reagents R_1 and R_2 to form coloured comlexes that can be measured at the recommnded λ_{max} for each metal complex. A calibration graph is constractued using standarad solutions (μg/ml) of Co^{2+}, Ni^{2+}, Cu^{2+} , Hg^{2+} and Ag^{+}. Under optimum conditions a linear relationship is obtained as shown in Fig's. (20-23) between the absorbance and concentration of metals within the range listed in Tables (7 and 8). The correlation coefficient, slops, intercepts, standard deviation, relative standard deviation, standard error, detection and quantification limits, molar absorptivity and ***Sandell***(116) sensitivity are calculated. The reproducibility of the method is determined by six replicate samples.

For more accurate analysis, ***Ringbom***(115) optimum concentration ranges are determined by plotting log [M] in μg/ml against transmittance percent (T%) as shown in Fig's. (20-23), the linear portion of the S-shaped curve gives accurate range of analysis and the results are recorded in Tables (7 and 8).

The results obtained indicate high sensitivity in microdetermination of Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^{+} ions. At the same time the molar absorptivity can be enhanced by addition of (2.0 ml of 0.5% v/v) Tween 20 in case of Ag^{+}-R_1 complex only, whereas on using reagent R_2 the addition of (2.0 ml of 0.5% v/v) Tween 20 in case of Ni^{2+} and Ag^{+} and (2.0 ml of 0.5% v/v) Tween 40 in case of Cu^{2+} and Hg^{2+} leades to increase their molar absorptivity.

b) Evaluation of the accuracy and precision of the proposed method

In order to determine the accuracy and precision of the proposed method, solutions containing different concentrations of the metal ions under investigation are prepared and analysed in six replicates. The analytical results obtained from this investigation are summarized in Tables (9 and 10). The relative standard deviations relative error and the recovery percent at 95% confidence level are calculated. The results can be considered as very satisfactory, at least for the level of concentration examined.

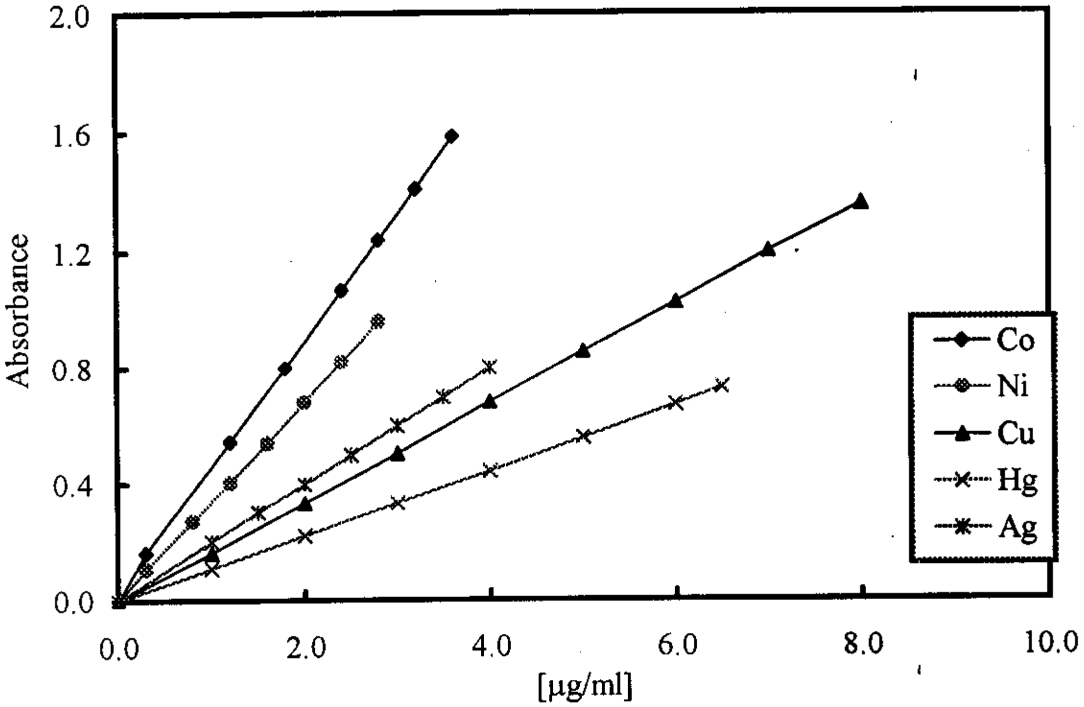
c) Interferences

One of the striking points of any new method is its interfering limit of the potential interferences that reduce the sensitivity of the proposed method. So to check and evaluate the tolerance limits of different interferes for the proposed method, the interferences due to several cations and anions are studied in detail. For this study, different amounts of the ionic species are added in the sample during the determination of metals under consedration. The tolerated limits are taken as those concentration causing change not greater than ±1% in the absorbance value. The results obtained showed that no interference is observed from the presence of Na^+, K^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Pb^{2+}, Bi^{3+}, Al^{3+}, La^{3+}, NH_4^+, HCO_3^- SO_4^{2-}, NO^-_3, VO^-_3, Mo^{6+}, W^{6+}, oxalate, tartarate, acetate, phosphate and borate. Whereas Zn^{2+}, Cd^{2+} and Fe^{3+} ions interfered, so it must be removed or masked.

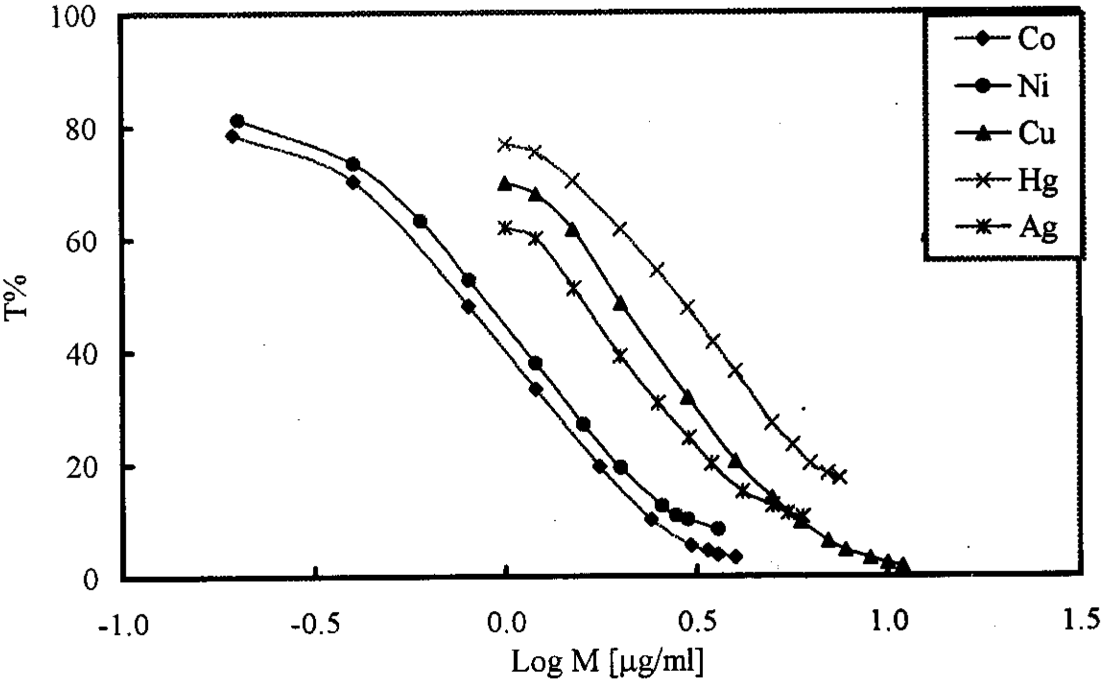
3.5.1.10. Analytical applications

In order to test the applicability of the proposed method, we apply the constructed model for determination of metal ions under investigation in real matrix samples. For this purpose, cyanocobamine injection for Co^{2+}, waste water for Hg^{2+} and two alloy samples (Ni-Cu and Cu-Ag alloys) are analyzed using the proposed method. The concentrations of of the studied metal ions in these samples are calculated from the appropriate calibration graph.The results of the prediction are summarized in Tables (11-12) and compared with the results obtained by atomic absorption method. The results obtained are compared statistically by the Student's t-value and variance ratio F-test with those obtained by atomic absorption method.

The results in Tables (11-12) show that the Student's t-value does not exceed the theoretical tabulated value indicating no significance difference between the methods compared. The F-values also show that no signficant difference between the the proposed method and the official method. The standard addition technique is applied and the results obtained in Tables (13-14) show that, the proposed method is highly sensitive and able to predict the concentrations of Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^{+} in the above real samples with high accuracy.

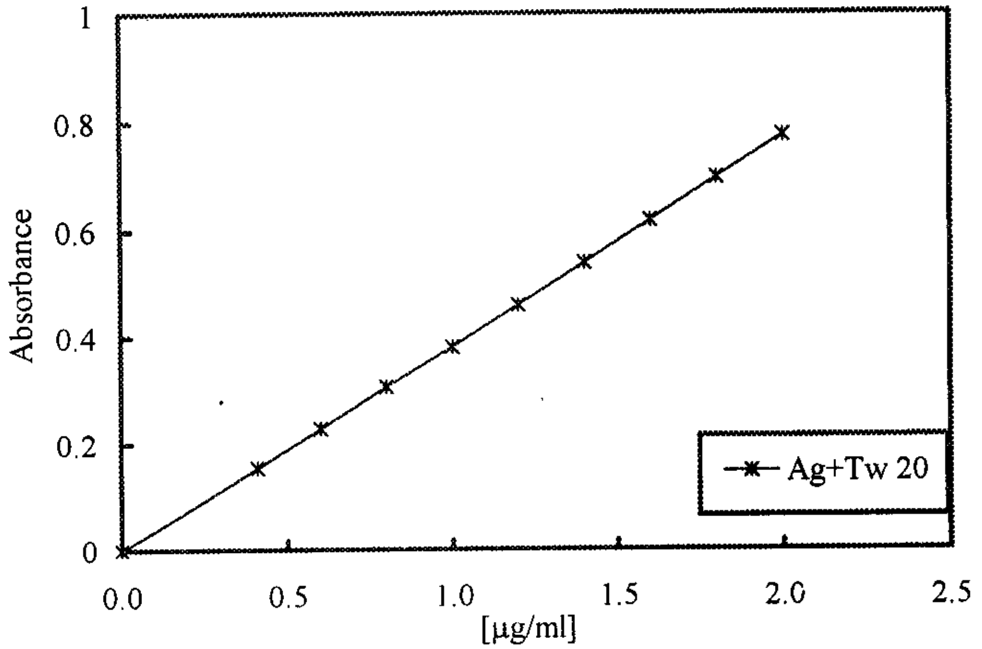


(a): Application of Beer's law for the studied complexes using the optimum volume (2.0×10^{-3} M) of reagent R_1

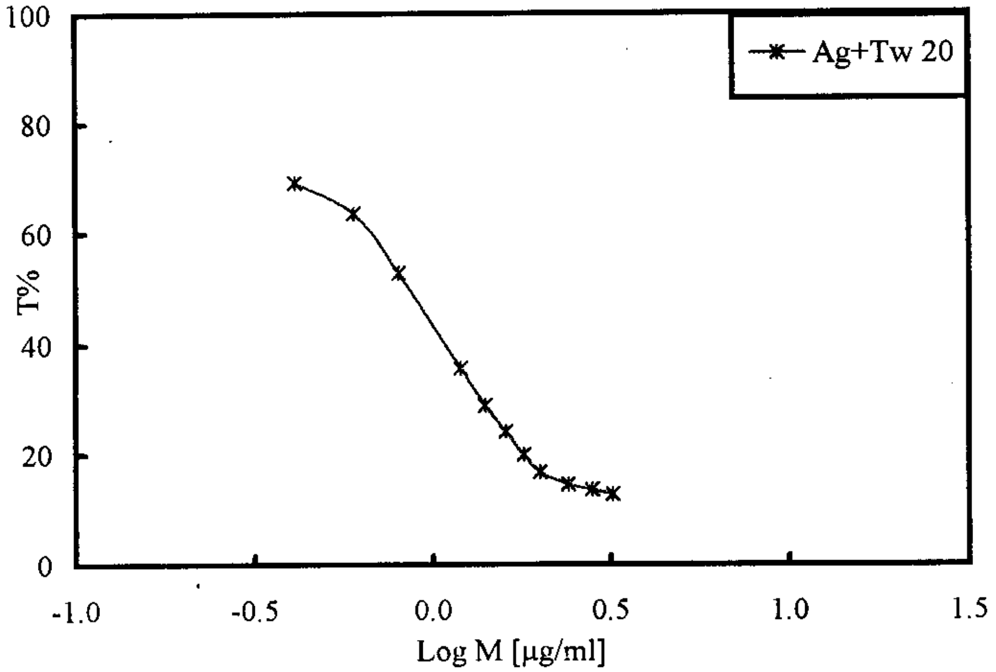


(b): Ringbom plots for the studied complexes with reagent R_1

Fig. (20)

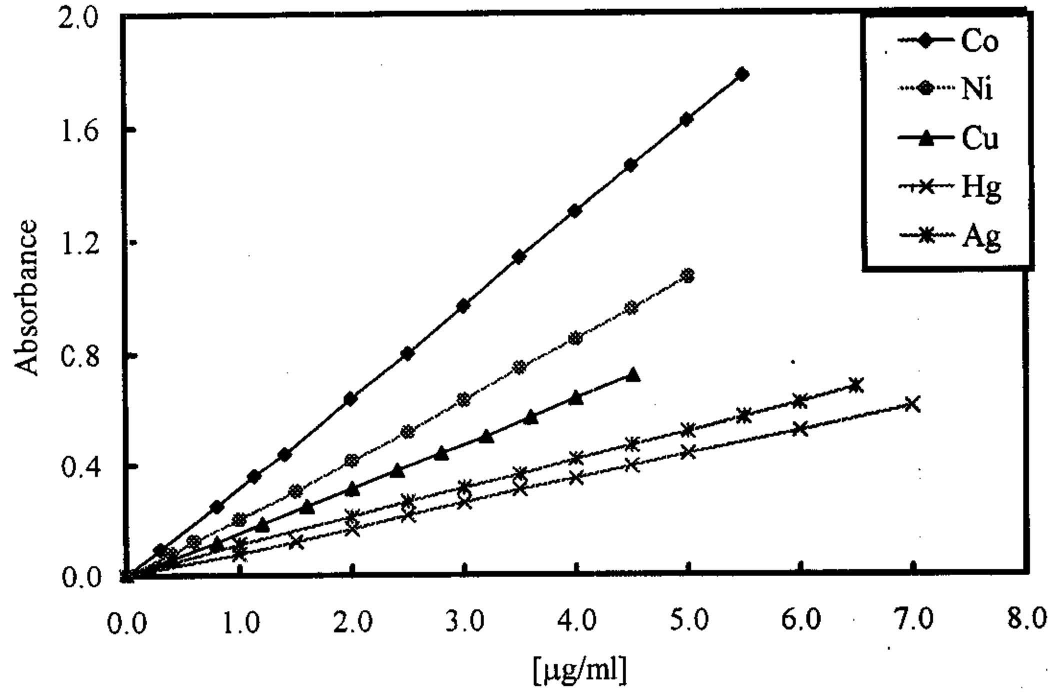


(a): Application of Beer's law for the studied complex using the optimum volume (2.0×10^{-3} M) of reagent R_1 in the presence of surfactant

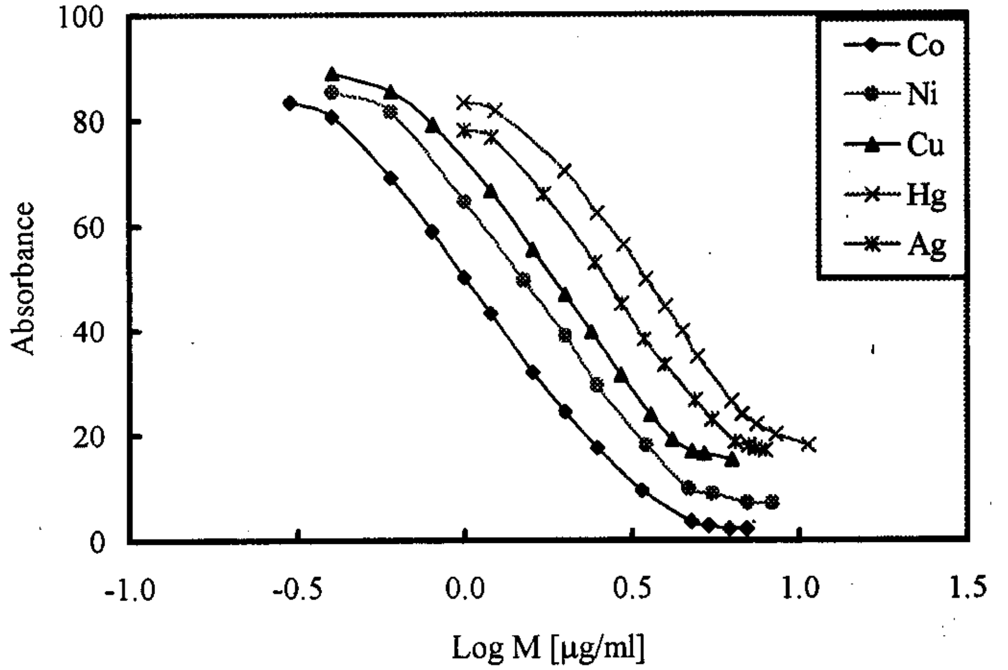


(b): Ringbom plots for the studied complex with reagent R_1 in the presence of surfactant

Fig. (21)

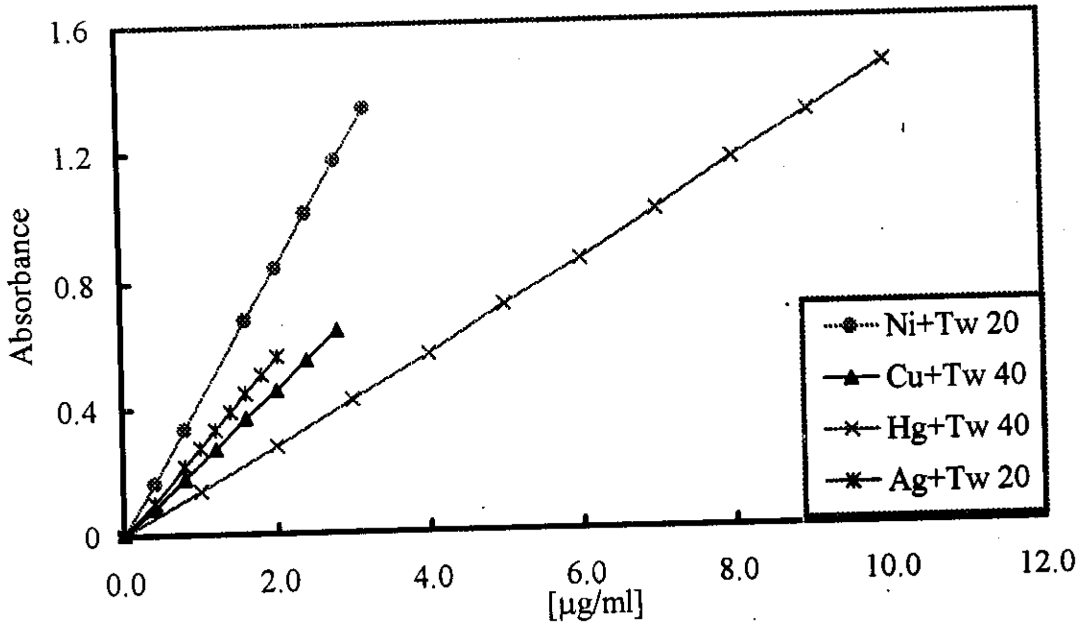


(a): Application of Beer's law for the studied metal ions complexes using the optimum volume (2.0×10^{-3} M) of reagent R_2

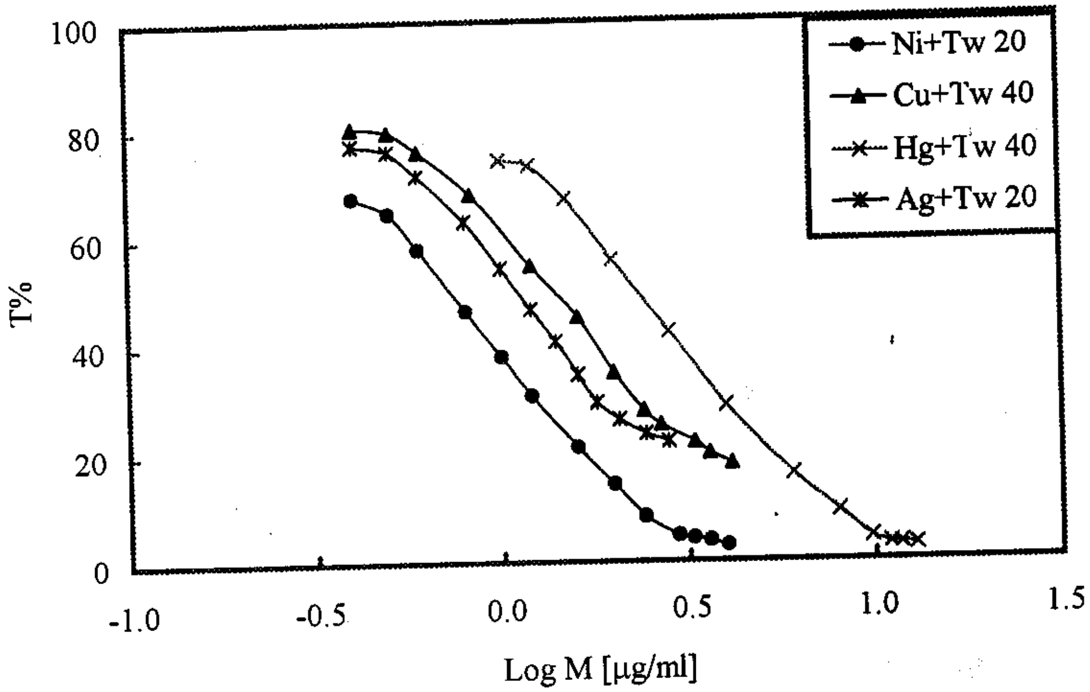


(b): Ringbom plots for the studied metal ions complexes using reagent R_2

Fig. (22)



(a): Application of Beer's law for the studied metal ions complexes using the optimum volume (2.0×10^{-3} M) of reagent R_2 in the presence surfactants



(b): Ringbom plots for the studied ions complexes using reagent R_3 in the presence of surfactants

Fig. (23)

Table (7): Analytical parameters of reagent R_1 complexes with Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^+ ions.

Parameters	**R_1**					
	Co^{2+}	**Ni^{2+}**	**Cu^{2+}**	**Hg^{2+}**	**Ag^+**	
					Without^S	**With^S**
pH	10.50	9.50	9.50	6.50	9.50	9.50
λ_{max} (nm)	540	534	542	552	524	531
Beer's range (μg/ml)	0.3-3.6	0.3-2.8	1.0-8.0	1.0-6.5	1.0-4.0	0.4-2.0
Rangbom rang (μg/ml)	0.4-3.4	0.4-2.6	1.2-7.8	1.2-6.2	1.2-3.7	0.5-1.8
Detection Limit (μg/ml)	0.036	0.044	0.112	0.127	0.124	0.029
Quantification limit (μg/ml)	0.119	0.147	0.372	0.423	0.416	0.097
Standard deviation*	0.0091	0.0054	0.0058	0.0071	0.0074	0.0083
Relative standard deviation	0.891	0.529	0.592	0.348	0.679	0.769
Error %	0.367	0.204	0.204	0.286	0.286	0.326
Slope	0.392	0.363	0.174	0.111	0.199	0.389
Intercept	-0.006	-0.002	-0.013	-0.003	-0.001	-0.004
Correlation Coefficient	0.9998	0.9999	0.9998	0.9998	0.9997	0.9999
Molar absorptivity x 10^4 ($l.\ mol^{-1}\ cm^{-1}$)	2.31	2.13	1.11	2.23	2.46	4.20
Sandell Sensitivity (ng cm^{-2})	2.55	2.75	5.75	9.01	5.03	2.57

*: Average of six determinations

^{S}: surfactant

Table (8) Analytical parameters of reagent R_2 complexes with Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^{+} ions

Parameters	**Co^{2+}**	**Ni^{2+}**		**Cu^{2+}**		**Hg^{2+}**		**Ag^{+}**	
		without S	**with S**	**Without S**	**with S**	**without S**	**with S**	**without S**	**with S**
pH	10.50	9.50	9.50	5.50	5.50	9.50	9.50	9.50	9.50
λ_{max} (nm)	547	540	550	555	560	560	565	531	543
Beer's range (µg/ml)	0.3-5.5	0.4-5.0	0.4-3.2	0.4-4.5	0.4-2.8	1.0-7.0	1.0-10.0	1.0-6.5	0.4-2.0
Ringbom Optimum range (µg/ml)	0.4-5.2	0.6-4.7	0.5-3.0	0.6-4.2	0.5-2.6	1.2-6.8	1.2-9.8	1.2-6.2	0.5-1.8
Detection Limit (µg/ml)	0.058	0.087	0.046	0.136	0.094	0.171	0.108	0.099	0.049
Quantification limit (µg/ml)	0.192	0.290	0.154	0.458	0.314	0.571	0.359	0.333	0.164
Standard deviation	0.0081	0.0083	0.0069	0.0074	0.0053	0.0079	0.0049	0.0061	0.0050
Relative standard deviation	0.770	0.762	0.660	0.352	0.505	0.267	0.126	0.239	0.950
Error %	0.326	0.326	0.290	0.286	0.204	0.326	0.204	0.245	0.204
Slope	0.336	0.214	0.410	0.160	0.231	0.087	0.146	0.104	0.286
Intercept	-0.009	-0.006	0.007	-0.004	-0.002	-0.001	-0.008	-0.002	-0.009
Correlation Coefficient	0.9999	0.9997	0.9999	0.9997	0.9998	0.9997	0.9998	0.9998	0.9997
Molar absorptivity x 10^4 (L. mol^{-1} cm^{-1})	1.98	1.25	2.41	1.02	1.47	1.75	2.93	1.12	3.08
Sandell Sensitivity (ng cm^{-2})	2.98	4.67	2.44	6.25	4.33	11.49	6.85	9.62	3.50

* Average of six determinations

^{S}: Surfactant

Table (9): Evaluation of the accuracy and precision of the proposed method using reagent R_1.

Sample	Taken (μg/ml)	Found* (μg/ml)	Recovery %	RSD %	Relative Error	Confidence limits**
$CoCl_2$	1.0	1.012	101.20	0.859	+1.20	1.012 ± 0.0091
	2.0	2.011	100.55	0.333	+0.55	2.011 ± 0.0070
$NiCl_2$	1.0	0.995	99.50	0.763	-0.50	0.995 ± 0.0079
	2.0	2.021	101.05	0.415	+1.05	2.021 ± 0.0088
$CuCl_2$	2.0	1.993	99.65	0.491	-0.35	1.993 ± 0.0103
	4.0	3.994	99.85	0.247	-0.15	3.994 ± 0.0104
$AgNO_3$	1.0	1.011	101.10	0.573	+1.10	1.011 ± 0.0061
		1.014^{S}	101.40^{S}	0.582^{S}	+1.40^{S}	1.014 ± 0.0062^{S}
	2.0	2.018	100.90	0.227	+0.90	2.018 ± 0.0048
		2.016^{S}	100.80^{S}	0.441^{S}	+0.80^{S}	2.016 ± 0.0093^{S}
$HgCl_2$	2.0	1.989	99.45	0.306	-0.55	1.989 ± 0.0064
	4.0	4.027	100.67	0.178	+0.67	4.027 ± 0.0076

*: Average of six determinations.

**: 95% confidence limits and five degrees of freedom.

^{S}: The calculated values in the presence of surfactants.

Table (10): Evaluation of the accuracy and precision of the proposed method using reagent R_2.

Sample	Taken (µg/ml)	Found* (µg/ml)	Recovery %	RSD %	Relative Error	Confidence limits**
$CoCl_2$	2.0	2.012	100.60	0.392	+0.60	2.012 ± 0.0083
	3.0	3.024	100.80	0.297	+0.80	3.024 ± 0.0094
$NiCl_2$	2.0	2.028	101.40	0.483	+1.40	2.028 ± 0.0103
		2.010^{S}	100.50^{S}	0.453^{S}	$+1.50^{s}$	2.010 ± 0.0096^{S}
	3.0	2.998	99.93	0.320	-0.07	2.998 ± 0.0101
		3.007^{S}	100.23^{S}	0.289^{S}	$+1.23^{S}$	3.007 ± 0.0091^{S}
$CuCl_2$	1.0	1.011	101.10	0.702	+1.10	1.011 ± 0.0075
		0.998^{S}	99.80^{S}	0.992^{S}	-0.20^{S}	0.998 ± 0.0104^{S}
	2.0	1.997	99.85	0.455	-0.15	1.997 ± 0.0096
		2.008^{S}	100.40^{S}	0.973^{S}	$+0.40^{S}$	2.008 ± 0.0099^{S}
$AgNO_3$	1.0	0.998	99.80	0.681	-0.20	0.998 ± 0.0071
		1.011^{S}	101.10^{S}	0.574^{S}	$+1.10^{S}$	1.011 ± 0.0061^{S}
	2.0	2.018	100.90	0.391	+0.90	2.018 ± 0.0083
		2.029^{S}	101.45^{S}	0.439^{S}	$+1.45^{S}$	2.029 ± 0.0093^{S}
$HgCl_2$	2.0	1.992	99.60	0.446	-0.40	1.992 ± 0.0093
		1.998^{S}	99.90^{S}	0.340^{S}	-0.10^{S}	1.998 ± 0.0071^{S}
	4.0	3.991	99.77	0.248	-0.33	3.971 ± 0.0104
		4.031^{S}	100.78^{S}	0.256^{S}	$+0.78^{S}$	4.031 ± 0.0108^{S}

*: Average of six determinations.

**: 95% confidence limits and five degrees of freedom.

S: The calculated values in the presence of surfactants.

Table (11): Evaluation of the accuracy and precision of the proposed method for determination of metal ions in real samples using reagent R_1.

Samples	Metal (%)	Metal Found (%)					
		AAS. method		Proposed method			
		Found* %	Recovery %	Found* %	Recovery %	t# test	F# value
Vitamine B_{12}	0.05	0.0502	100.40	0.0501	100.20	1.74	2.63
Ni^{2+} in (Ni-Cu alloy)	50	50.101	100.20	49.981	99.96	1.23	2.88
Cu^{2+} in (Ni-Cu alloy)	50	50.301	100.60	50.122	100.24	1.42	2.40
Cu^{2+} in (Ag-Cu alloy)	20	19.962	99.81	19.982	99.91	0.61	3.91
Ag^{2+} in (Ag-Cu alloy)	80	79.983	99.98	80.043 80.021^S	100.05 100.03^S	0.70 0.64^S	2.21 3.21^S
Hg^{2+} in waste water	0.156	0.157	100.64	0.158	101.28	1.09	2.14

*: Average of six determinations.

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

S: The calculated values in the presence of surfactant

Table (12): Evaluation of the accuracy and precision of the proposed method for determination of metal ions in real samples using reagent R_2.

Samples	Metal (%)	Metal Found (%)					
		AAS. method		Proposed method			
		Found* %	Recovery %	Found* %	Recovery %	t$^{\#}$ test	F$^{\#}$ value
Vitamine B_{12}	0.05	0.0502	100.40	0.0503	100.60	1.18	2.06
Ni^{2+} in (Ni-Cu alloy)	50	50.101	100.20	50.211	100.42	0.96	2.65
				50.152^{S}	100.30^{S}	1.11^{S}	2.78^{S}
Cu^{2+} in (Ni-Cu alloy)	50	50.302	100.60	50.091	100.18	1.09	2.10
				50.113^{S}	100.23^{S}	1.15^{S}	2.81^{S}
Cu^{2+} in (Ag-Cu alloy)	20	19.962	99.81	20.214	101.07	1.89	3.84
				20.221^{S}	101.11^{S}	1.71^{S}	3.61^{S}
Ag^{2+} in (Ag-Cu alloy)	80	79.983	99.98	79.963	99.95	1.32	3.38
				80.132^{S}	100.17^{S}	1.24^{S}	3.14^{S}
Hg^{2+} in waste water	0.156	0.157	100.64	0.1563	100.19	0.99	2.44
				0.1565^{S}	100.32^{S}	0.89^{S}	2.67^{S}

*: Average of six determinations.

$^{\#}$: Theoretical values for t-and F- values for five dgree of freedom and 95% confidence limits are 2.57 and 5.05, respectively.

^{S}: The calculated values in the presence of surfactant

Table (13): Determination of the studied metal ions in real samples applying the standard addition techinque using reagent R_1.

Sample	Taken (µg/ml)	Added (µg/ml)	Found* (µg/ml)		Recovery %	
Co^{2+} in (vitamine B_{12})	1.0	--	0.99		99.00	
		1.0	2.01		100.50	
		1.5	2.48		99.20	
		2.0	3.02		100.66	
Ni^{2+} in (Ni-Cu alloy)	1.0	--	0.98		98.00	
		0.5	1.52		101.33	
		1.0	2.0		101.00	
		1.5	2.49		99.60	
Cu^{2+} in (Ni-Cu alloy)	3.0	--	3.01		100.33	
		2.0	5.01		100.20	
		3.0	4.98		99.67	
		4.0	7.02		100.29	
Cu^{2+} in (Ag-Cu alloy)	3.0	--	3.01		100.33	
		2.0	5.03		100.60	
		3.0	6.01		100.17	
		4.0	6.98		99.71	
Ag^{+} in (Ag-Cu alloy)	0.5	--	0.51	0.49^{S}	102.00	98.00^{S}
		0.5	1.01	1.02^{S}	101.00	102.00^{S}
		1.0	1.49	1.51^{S}	99.33	100.67^{S}
		1.5	1.99	2.01^{S}	99.50	100.50^{S}
Hg^{2+} in waste water	3.0	--	2.97		99.00	
		1.0	4.02		100.50	
		2.0	4.04		100.80	
		3.0	5.97		99.50	

*: Average of six determinations

S: The calculated values in the presence of surfactant

Table (14): Determination of the studied metal ions in real samples applying the standard addition techinque using reagent R_2.

Sample	**Taken (µg/ml)**	**Added (µg/ml)**	**Found* (µg/ml)**		**Recovery %**	
Co^{2+} in (vitamine B_{12})	3.0	--	2.98		99.33	
		1.0	3.95		98.75	
		1.5	4.52		100.44	
		2.0	5.04		100.80	
Ni^{2+} in (Ni-Cu alloy)	1.0	--	0.99	1.01^{S}	99.00	101.00^{S}
		0.5	1.49	1.50^{S}	99.33	100.00^{S}
		1.0	1.98	1.99^{S}	99.00	99.50^{S}
		1.5	2.52	2.54^{S}	100.80	101.6^{S}
Cu^{2+} in (Ni-Cu alloy)	1.0	--	1.01	1.02^{S}	101.00	102.00^{S}
		0.5	1.51	1.53^{S}	100.66	102.00^{S}
		1.0	2.03	2.01^{S}	101.50	100.50^{S}
		1.5	2.48	2.52^{S}	99.20	100.80^{S}
Cu^{2+} in (Ag-Cu alloy)	1.0	--	1.01	0.99^{S}	101.00	99.00^{S}
		0.5	1.52	1.49^{S}	101.33	99.33
		1.0	1.98	2.03^{S}	99.00	101.50
		1.5	2.49	2.48^{S}	99.60	99.20
Ag^{+} in (Ag-Cu alloy)	0.5	--	0.49	0.50^{S}	98.00	100.00
		0.5	0.99		99.00	
		1.0	1.52		101.33	
		1.5	2.01		100.50	
Hg^{2+} in waste water	3.0	--	2.98	3.01^{S}	99.33	100.33^{S}
		2.0	4.97	5.02^{S}	99.40	100.40^{S}
		3.0	6.03	6.04^{S}	100.50	100.66^{S}
		4.0	7.04	6.99^{S}	100.57	99.86^{S}

*: Average of six determinations

^{S}: The calculated values in the presence of surfactant

3.5.2. Spectrophotometric studies of [2-amino-4-(m-tolyazo) pyridine-3-ol] R_3, [2-amino-4-(4-chlorophenylazo) pyridine-3-ol] R_4 complexes with Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^{+} ions

To establish the optimum conditions for the maximum complex formation, the influence of several experimental variables are studied and reported below.

3.5.2.1. Effect of pH

In order to establish the optimum pH value for each complex formed, between the ions Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^{+} with reagents R_3 and R_4 which dissolved in DMF is carried out in universal buffer solution of pH ranges (2.50-11.50). The absorbance of each complex prepared in 10 ml measuring flask is measured against a reagent blank solution.The relation between the absorption spectra of metal complexes at different pH values are obtained as shown in Fig's. (24 and 25).

The careful investigation of these figures shows that, the alkaline medium (pH 9.50-10.50) is suitable for Co^{2+}, Ni^{2+} and Ag^{+} complexes with the reagents R_3 and R_4, while the acidic medium (pH 5.50) is favoured in case of Cu^{2+} and Hg^{2+} complexes with the same reagents.

Furthermore, the optimum volume of the selected buffer is studied by using different volumes (1.0,...., 6.0 ml) of the selected pH in the sample and blank. The results show that 2.0 ml of the selected buffer is sufficient for complex formation as shown in Fig's. (24 and 25).

3.5.2.2. Determination of maximum wavelength of complex species

The maximum wavelengths of the metal ion complexes with R_3 and R_4 are investigated in universal buffer solution. Representative curves of Hg^{2+} and Ni^{2+} complexes with R_3 and R_4 respectively, are shown as in Fig.(26).

The curves (A-D) in Fig. (26-a) show that, at pH 5.50, reagent R_3 is absorbed maximally at 490 nm with buffer as a blank (curve A), whereas the complex of Hg^{2+} shows absorption band at 520 nm with buffer as a blank (curve B). When reagent R_3 used as a blank, the Hg^{2+} complex absorbes maximally at 560 nm (curve C). The addition of 1.0 ml of 0.5% (v/v) Tween 80 increases the absorbance and shifts the λ_{max} to 566 nm (curve D). The results show also that the addition of 1.0 ml of 0.5% (v/v) Tween 80 increases the absorbance and shifts the λ_{max} of Ag^+ complex. The complexes of Co^{2+}, Ni^{2+} and Cu^{2+} show no response for the surfactants and their λ_{max} are recorded in Table (15).

Fig. (26-b) showed the absorption spectra of the buffered solution of reagent R_4 in absence and in presence of Ni^{2+}. Inspection of each spectrum shown in the figure indicates that the reagent absorbes maximally at 473 nm at pH 9.50 with buffer as a blank (curve A), while the Ni^{2+} complex shows absorption band at 485 nm with buffer as a blank (curve B). Using R_4 and buffer as a blank, the complex of Ni^{2+} ion is absorbed at 535 nm (curve C). The addition of 1.5 ml of 0.5% (v/v) Tween 40 increases the absorbance and shifts the λ_{max} (curve D).

Another investigation of the effect of surfactants on the complexation prosess of reagent ***R₄*** shows that, in addition of Ni^{2+}, only the complexation with Ag^{+} is affected with the addition of 1.5 ml of 0.5% (v/v) Tween 40 that increases the absorbance of such complex and shifts its λ_{max}. The complexes of Co^{2+}, Cu^{2+} and Hg^{2+} with ***R₄*** are not affected by the addition of surfactants and their λ_{max} values are recorded in Table (16).

3.5.2.3. Effect of surfactant

To increase the sensitivity of the metal complexes formed, different concentrations (0.5, 1.0,..., 3.0 ml of 0.5% v/v or w/v) from different surfactants are added to fixed concentration (1.0 ml of 2.0 x 10^{-3} M) of the reagent and (1.0 ml of 1.0 x 10^{-3} M) of metal ion in the presence of 2.0 ml of the selected pH in 10 ml measuring flask. The volume is completed to the mark with bidistilled water.

By measuring the absorbance of each complex it is found that, 1.0 ml of 0.5% (v/v) Tween 80 increases the absorbance of Hg^{2+} and Ag^{+} complexes with ***R₃*** and causes shift in their λ_{max} while the complexes of Co^{2+}, Ni^{2+} and Cu^{2+} ions are not affected by the addition of the surfactants. Using reagent ***R₄***, the absorbance of Ni^{2+} and Ag^{+} complexes are increased and the λ_{max} values are red shifted by 10 and 9 nm for Ni^{2+} and Ag^{+} respectively, by the addition of 1.5 ml of 0.5% (v/v) Tween 40. Other metal ion complexes are not affected by the addition of any surfactant.

3.5.2.4. Effect of reagent concentration

When various concentrations of reagent (0.2, 0.5,...., 2.5 ml of 2.0 x 10^{-3} M) are added to fixed concentration of metal ion (1.0 ml of 1.0 x 10^{-3} M) in the presence of 2.0 ml of the selected pH and optimum amount of surfactants in 10 ml measuring flask completed by distilled water (in case of metals that have sensitivity for surfactants). The absorbance is measured against reagent blank at the recommended λ_{max} as shown in Fig's. (27 and 28).

It is found that, 1.7 ml of R_3 or R_4 is sufficient in case of Co^{2+} and Ni^{2+} complexes, whereas 1.0 ml of the same reagents is enough for Cu^{2+} complexes. In case of Hg^{2+} complexes with R_3 or R_4 have mximum absorbance value at 1.5 ml and 1.0 ml respectively, while 1.5 ml of the same reagents in case of Ag^+ are sufficient for the production of maximum and reproducible colour intensity in using R_3 or R_4, respectively. The optimum reagent concentration with the metal ions that have sensitivity to the presence of surfactants is obtained and recorded in Tables (15 and 16).

3.5.2.5. Effect of time and temperature

The time required for complete colour development of the complexes formed between reagents R_3 and R_4 with metal ions under investigation is studied. Allowing the reactants to stand at various time intervals, it is observed that the time has no effect on the maximum colour intensity. The complexes formed are found to be stable for 24 h except for Cu^{2+} and Hg^{2+} complexes with R_4 that show stability for 10 h. Also no change in the absorbance occurred on increasing the temperature up to 60 °C after which the absorbance is fade slowly.

3.5.2.6. Effect of sequence of addition

Investigation of sequence of addition is illustrated by measuring the spectrum of the sample solution that contains reagent, metal, buffer and surfactant in different order against blank solution prepared by the same way without metal ion. The results show that the order (reagent-buffer-surfactant-metal) gives high absorbance value for the metal ions that show a sensitivity for the presence of surfactants, while the order (reagent-buffer-metal) is favoured for all metal ions in the absence of surfactants.

3.5.2.7. Molecular structure of metal complexes

The stiochiometric ratios of the formed complexes between Co^{2+}, Ni^{2+} and Cu^{2+} , Hg^{2+} and Ag^{+} ions and reagents $\boldsymbol{R_3}$ and $\boldsymbol{R_4}$ are studied by continuous variation and molar ratio methods. The treatment of the spectral data obtained from the previous methods is extended to evaluate the stability constants of the formed metal complexes.

a) Molar ratio method

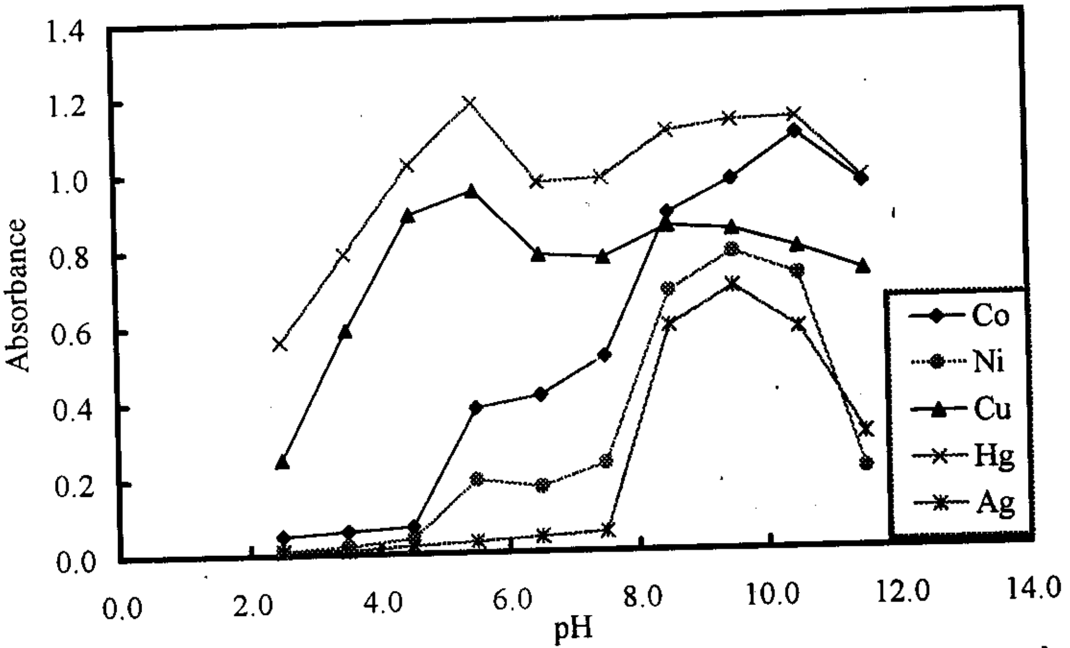
By using the molar ratio method, different concentration of the reaent $\boldsymbol{R_3}$ or $\boldsymbol{R_4}$ (0.2, 0.4,..., 2.4 ml of 1.0×10^{-3} M) are added to 2.0 ml solution at the selected pH and (0.5 ml of 1.0×10^{-3} M) of metal ion. The volume is completed to 10 ml with bidistilled water and the absorbance is measured against reagent blank solution. For the metal ions that have sensitivity for the addition of surfactants, the method is repeated with the addition of optimum amount of 0.5% (v/v) of surfactant in both sample and blank. The resulted absorbance is plotted against the molar ratio [R/M] as shown in Fig's. (29-32). The results obtained reveals the formation of 2:1 (R:M) ratio of all metal complexes.

b) Continuous variation method

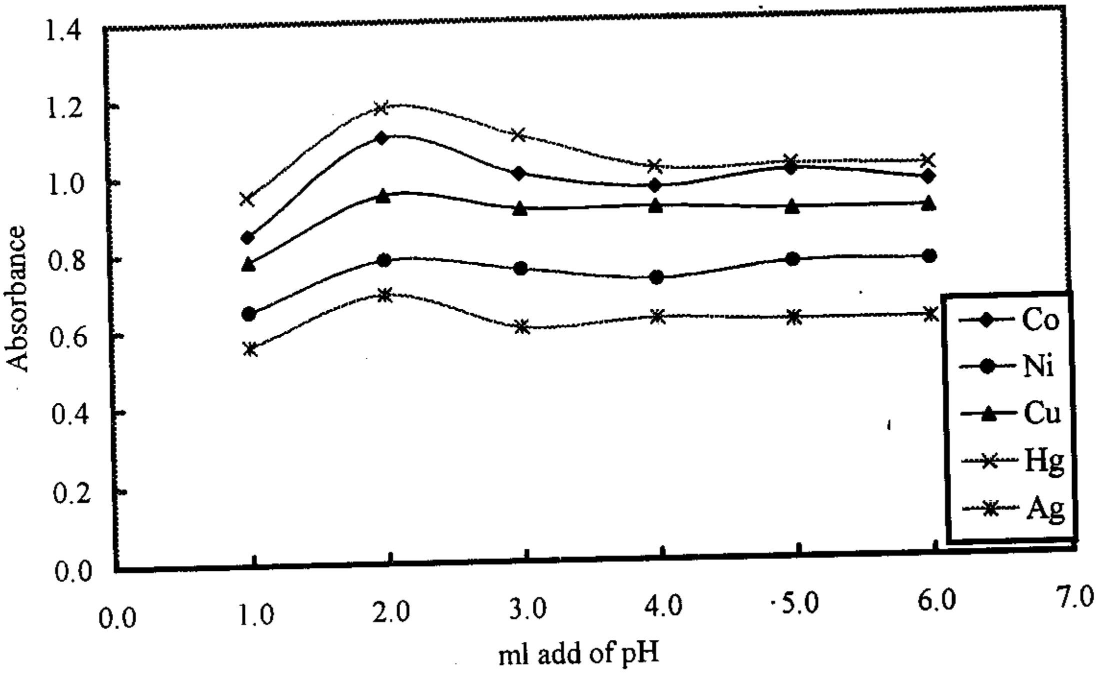
The molecular ratio of the complexes formed between reagent R_3 or R_4 and the metal ions can be studied by using the continuous variation method in which the total molar concentration of the reagent and the metal ion is kept constant at (2.0×10^{-4} M) in the partial assay solution. For the metal ions that have sensitivity for the presence of surfactants, the procedure is repeated with the addition of the optimum amount of surfactant in both sample and blank. The absorbance is then plotted against the mole fraction of the reagent as shown in Fig's. (29-32). The results obtained by this method show agreement with those obtained by the molar ratio method.

3.5.2.8. Stability constant of the formed complexes

The spectrophotometric methods that are usually applied to establish the stoichiometry of the metal ion complexes can also be used for the determination of their stability constants in solution applying ***Issa***(114) method. Careful investigation of the results obtained shows that, the stability constants of Hg^{2+} and Ag^{+} complexes with reagent R_3 are increased by the addition of 1.0 ml of 0.5% (v/v) Tween 80. Also the stability constants of Ni^{2+} and Ag^{+} complexes with reagent R_4 are increased by the addition of 1.5 ml of 0.5% (v/v) Tween 40 in comparison with the same complexes in absence of surfactant.

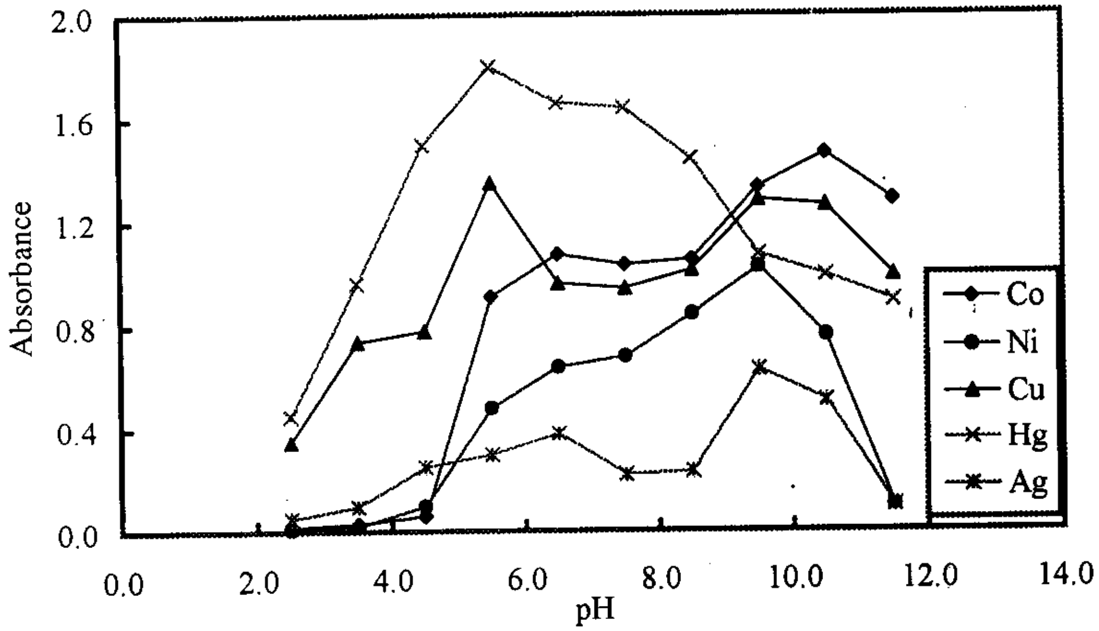


(a): Effect of pH on the maximum absorbances of metal ions complexes using (2.0×10^{-3} M) of reagent R_3

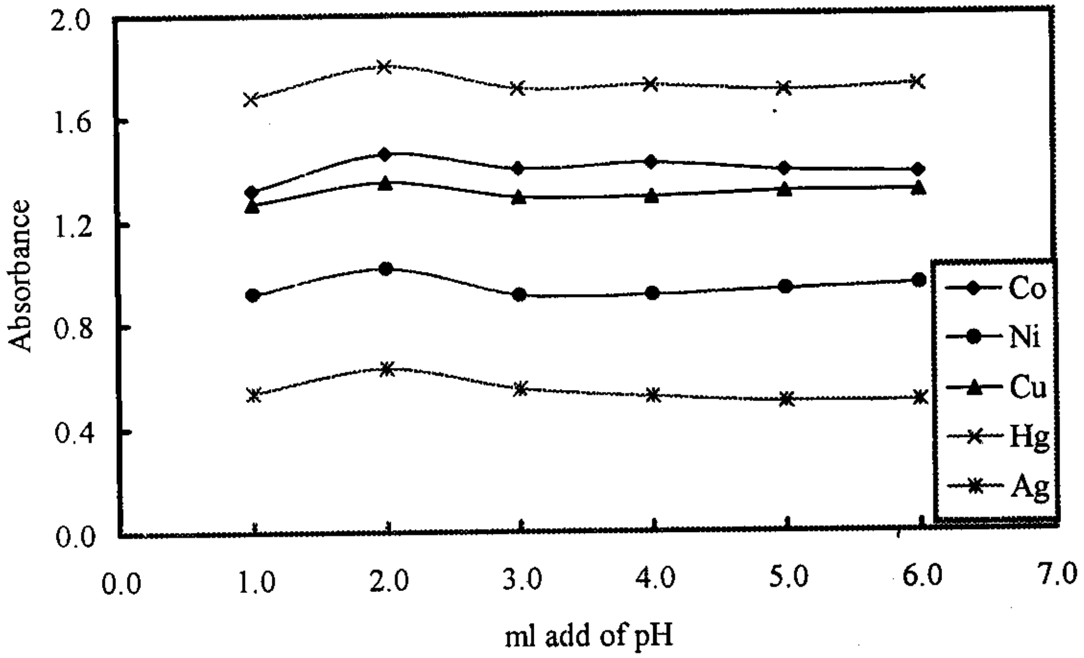


(a): Effect of ml added of buffer solution of the recommended pH on the complex formation using (2.0×10^{-3} M) of reagent R_3

Fig. (24)

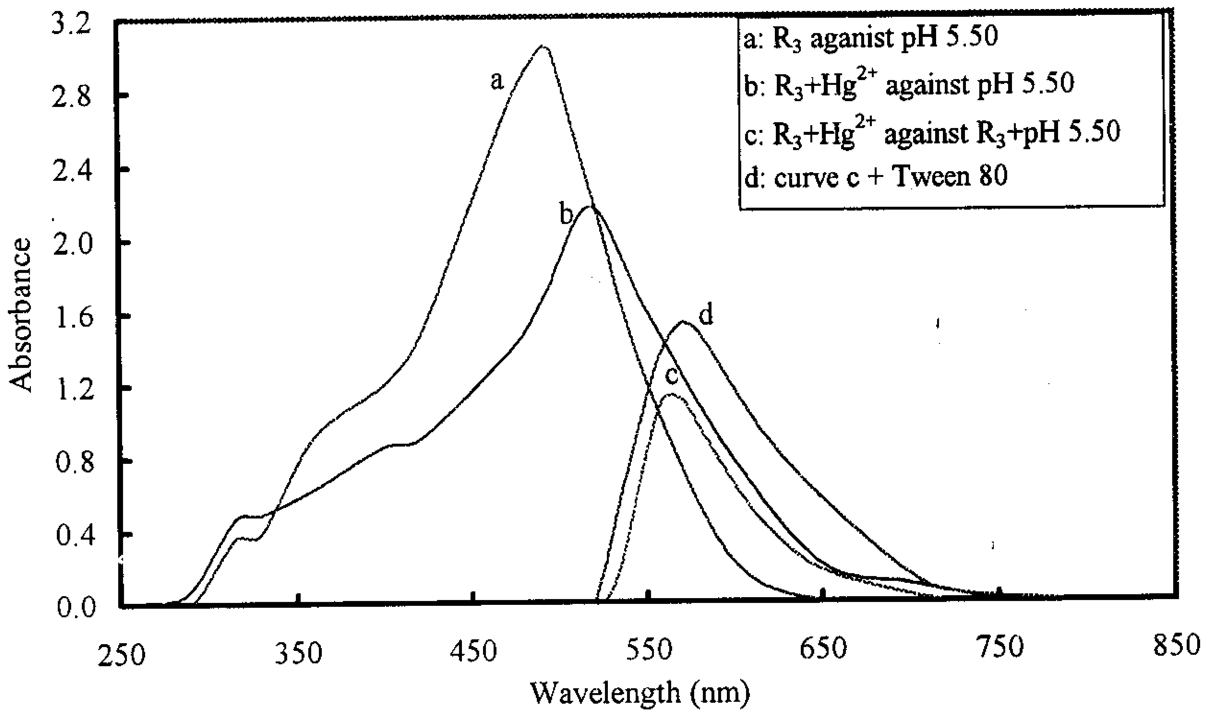


(a): Effect of pH on the maximum absorbances of metal ions complexes using (2.0 x 10-3 M) of reagent R_4

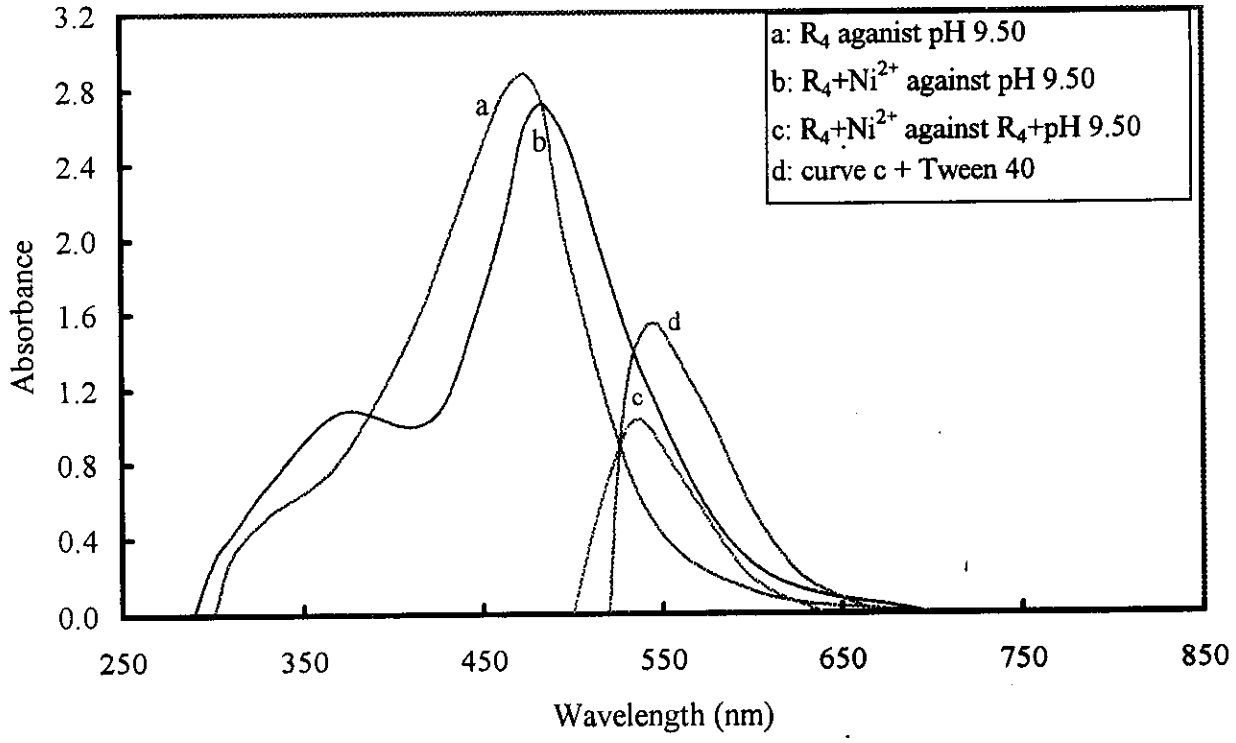


(b): Effect of ml added of buffer solution of the recommended pH on the complex formation using (2.0 x 10^{-3} M) of reagent R_4

Fig. (25)

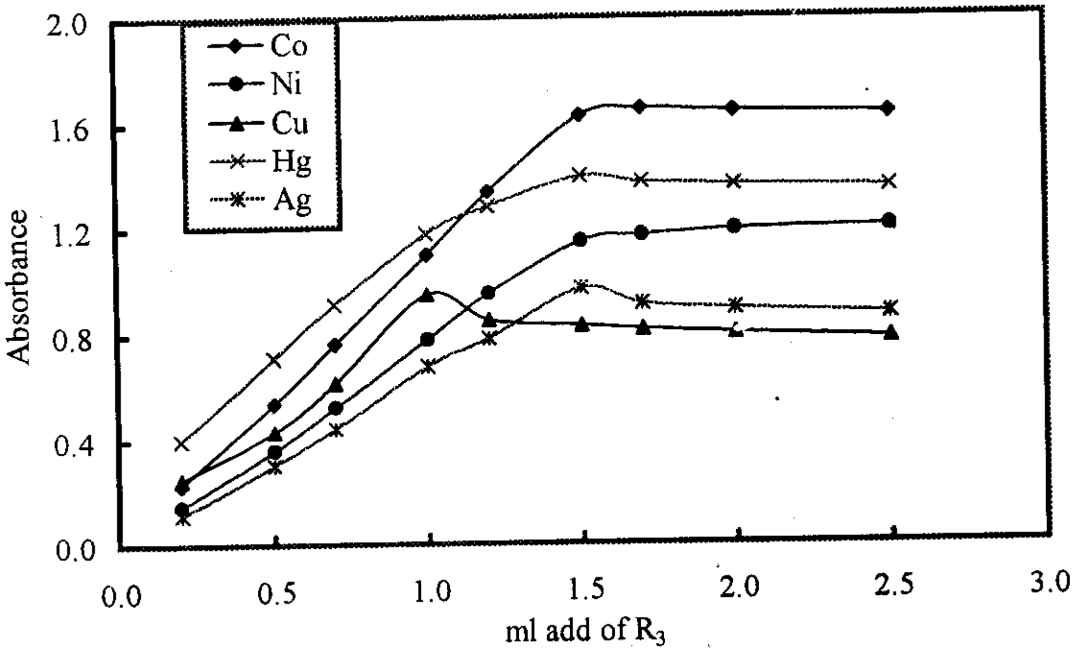


(a): Absorption spectra of (2 .0 x 10^{-3} M) R_3 with (1.0 x 10^{-3} M) of Hg^{2+} in the presence of (1.0 ml 0.5% v/v) Tween 80

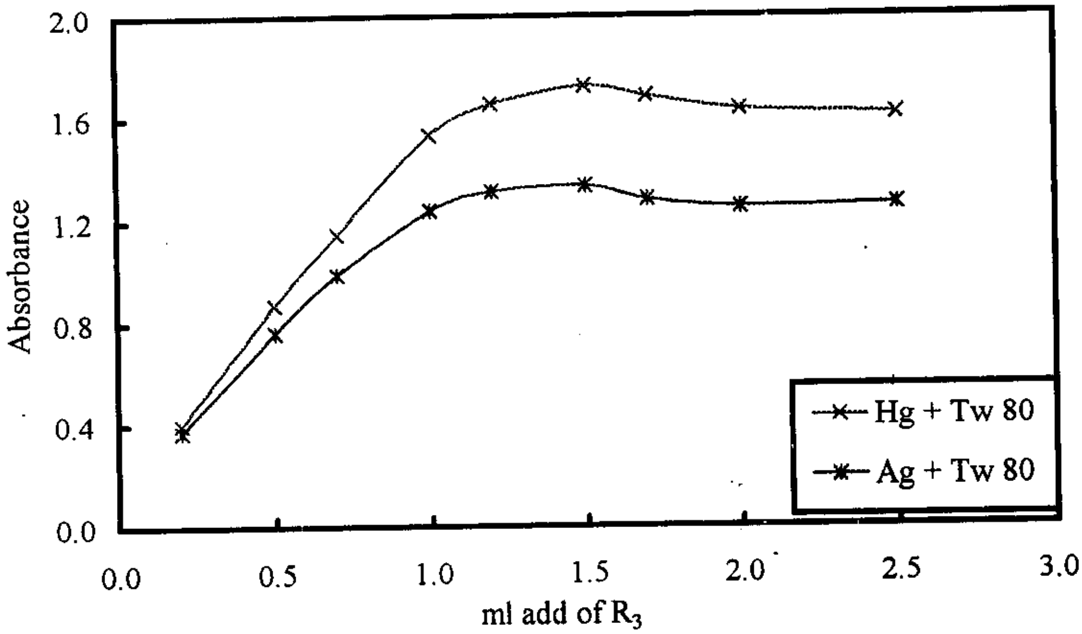


(b): Absorption spectra of (2 .0 x 10^{-3} M) R_4 with (1.0 x 10^{-3} M) of Ni^{2+} in the presence of (1.5 ml 0.5% v/v) Tween 40

Fig. (26)

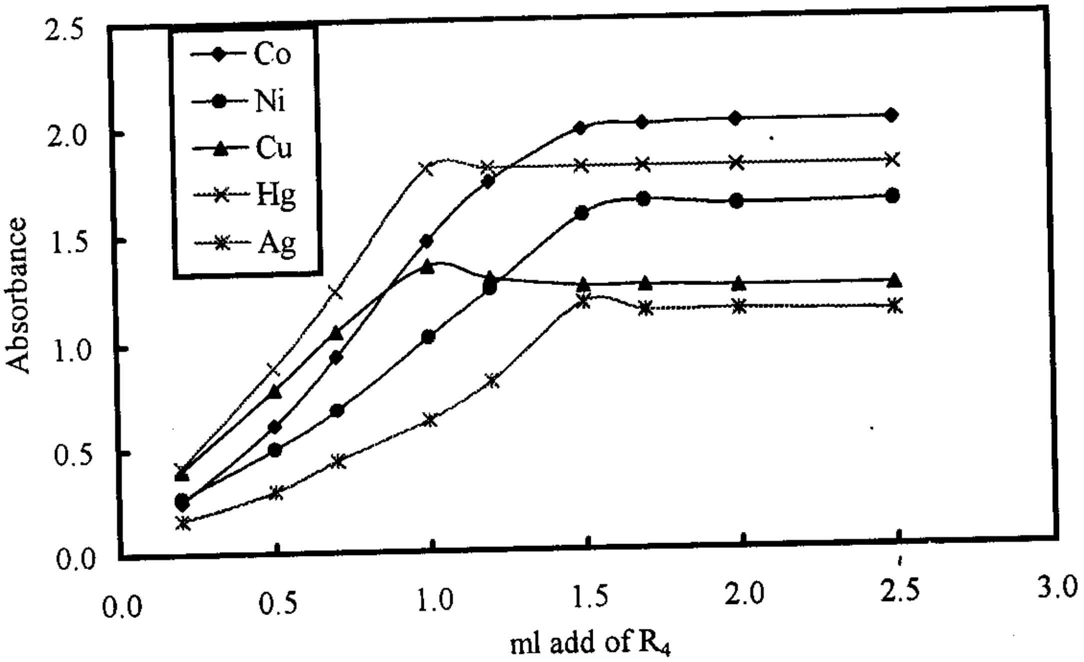


(a): Effect of reagent concentration on the absorbance of metal ions complexes using (2.0×10^{-3} M) of reagent R_3

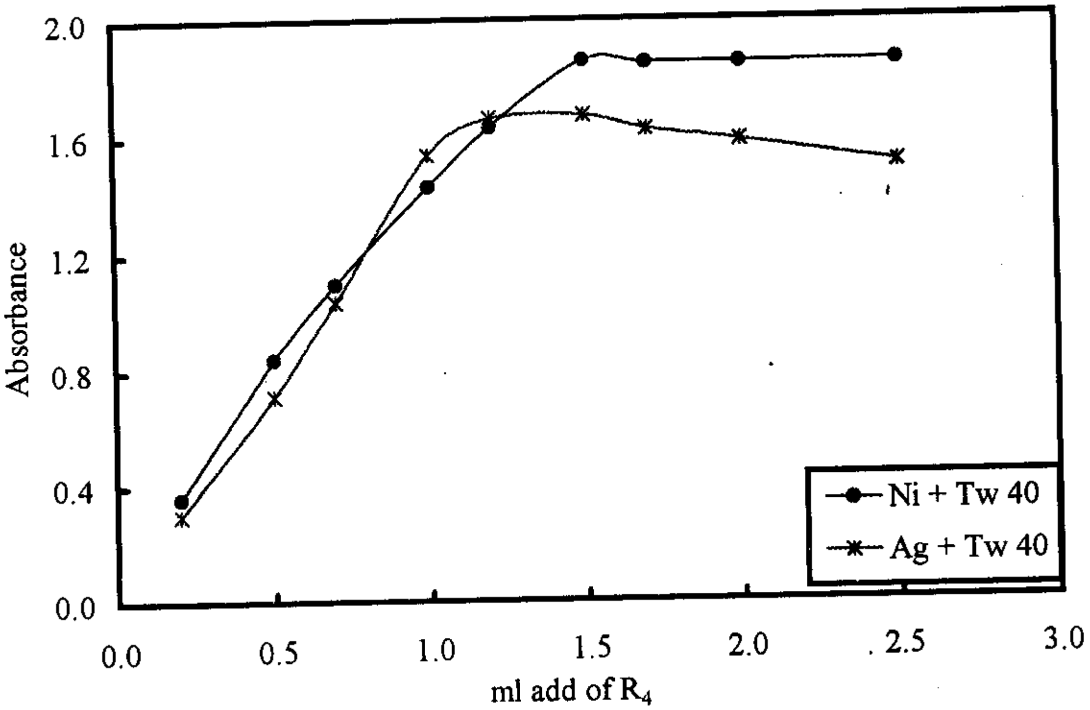


(b): Effect of reagent concentration on the absorbance of metal ions complexes in the presence of surfactant using (2.0×10^{-3} M) of reagent R_3

Fig. (27)

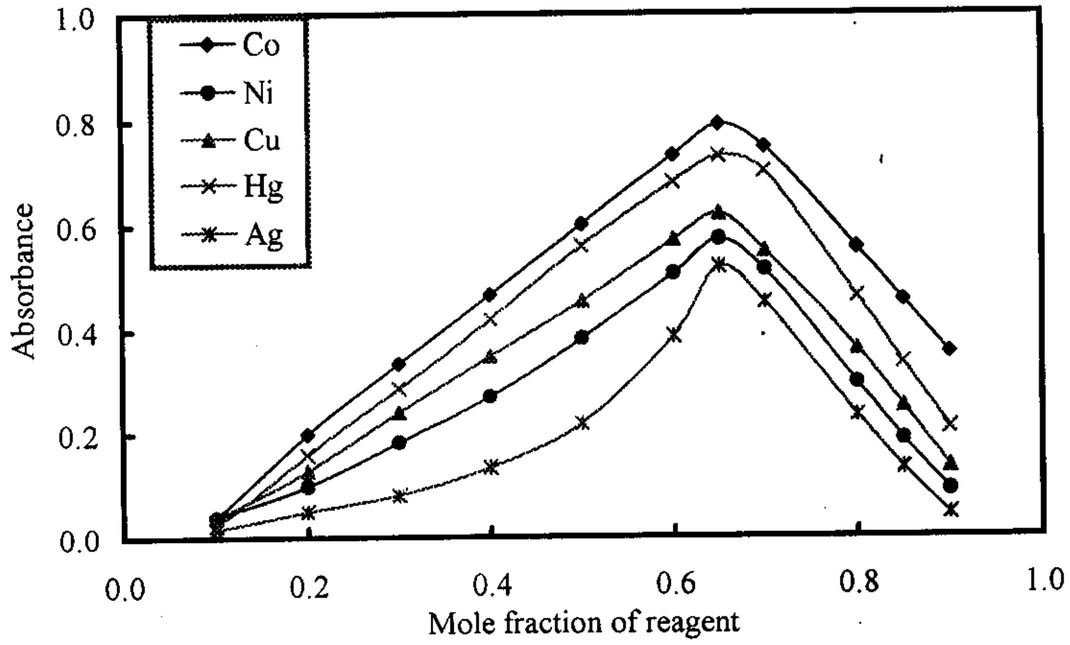


(a): **Effect of reagent concentration on the absorbance of metal ions complexes using (2.0 x 10^{-3} M) of reagent R_4**

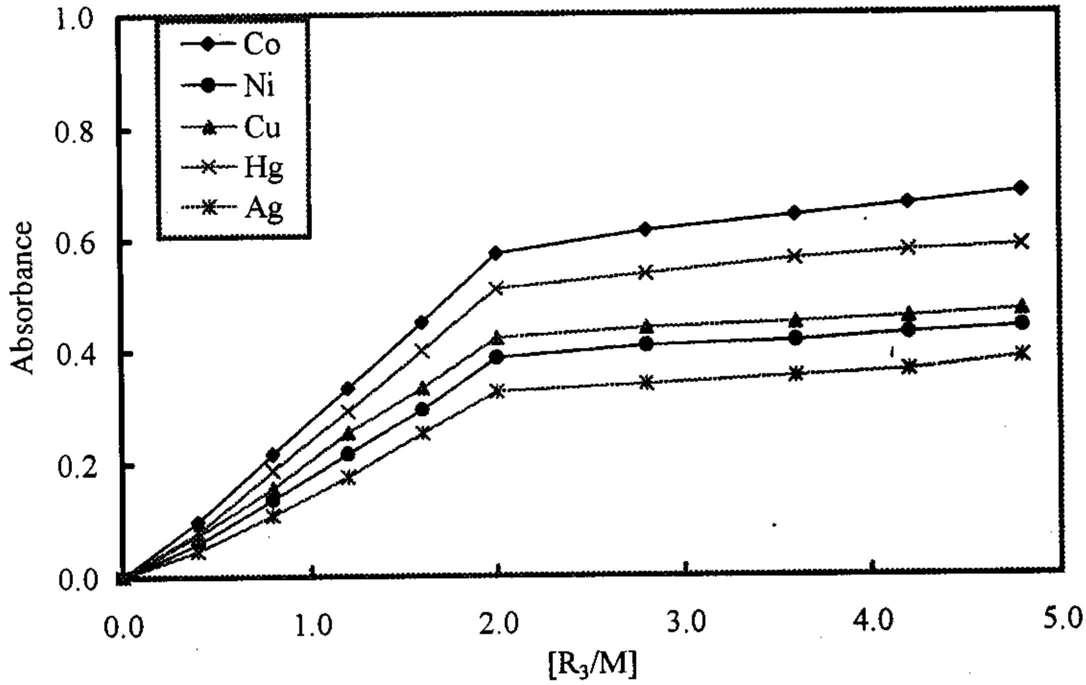


(b): **Effect of reagent concentration on the absorbance of metal ions complexes in the presence of surfactant using (2.0 x 10^{-3} M) of reagent R_4**

Fig. (28)

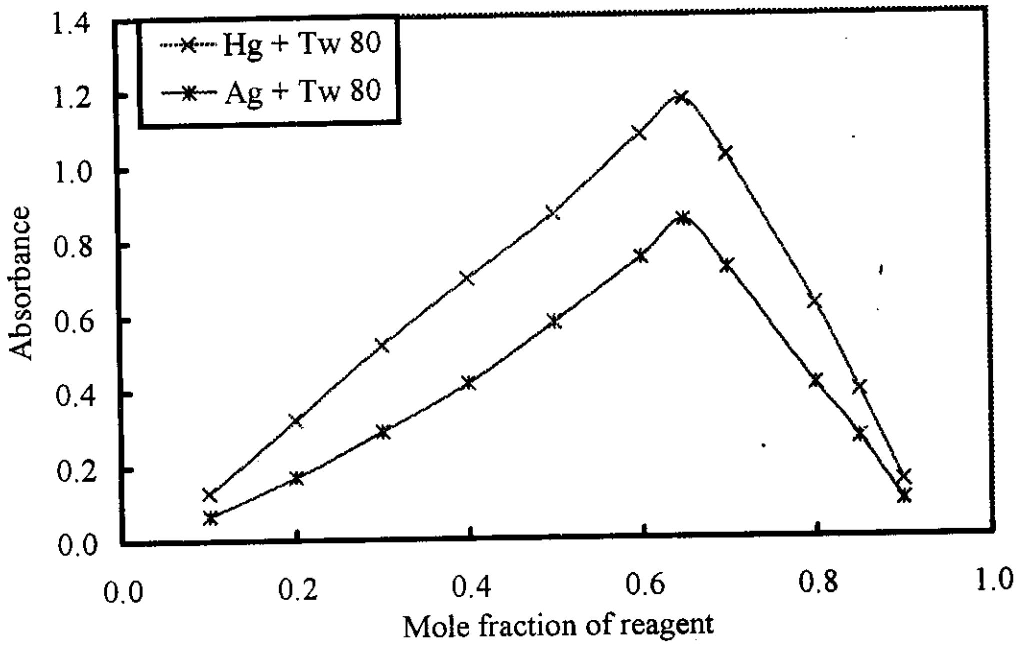


(a): Continuous variation using (1.0 x 10^{-3} M) of reagent R_3 with (1.0 x 10^{-3} M) of metal ions

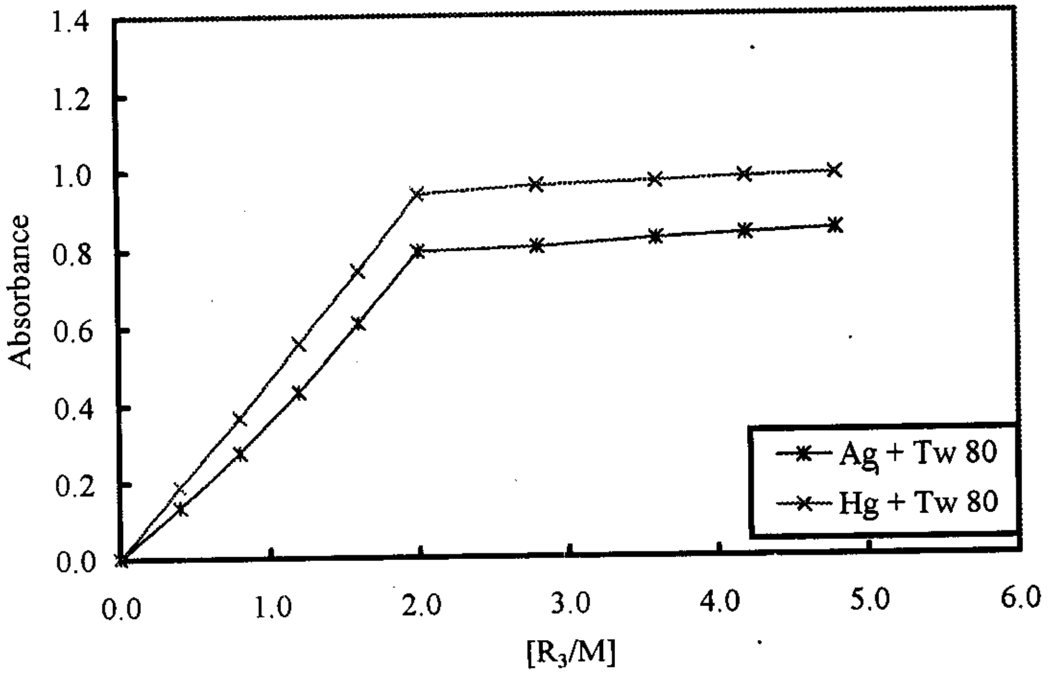


(b): Molar ratio using (1.0 x 10^{-3} M) of reagent R_3 with (0.5 ml of 1.0 x 10^{-3} M) of metal ions

Fig. (29)

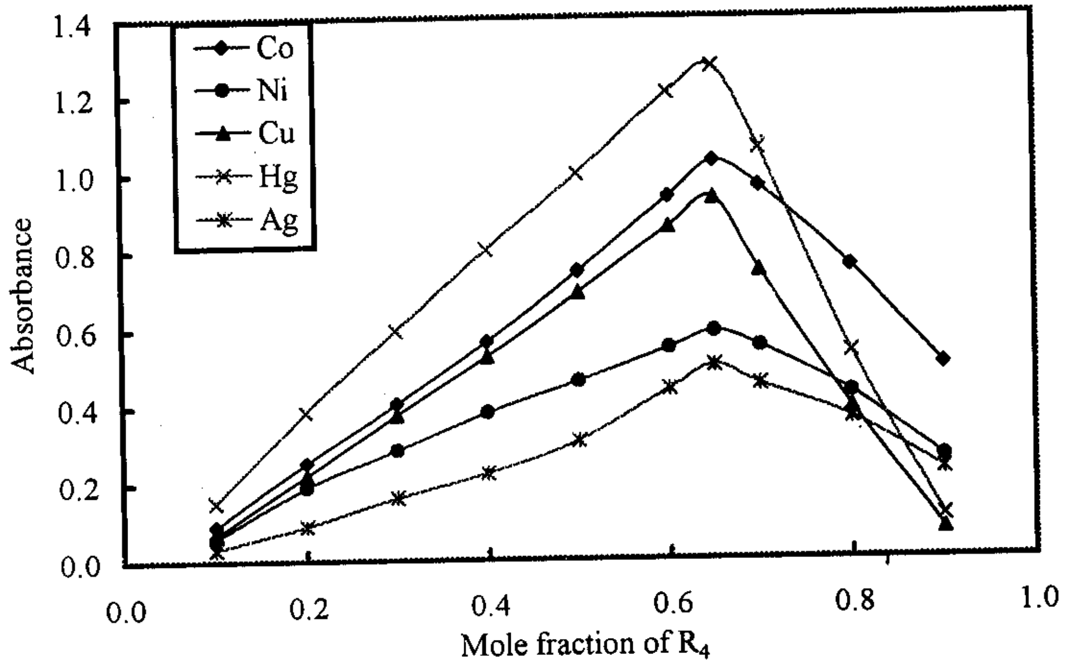


(a): Continuous variation using (1.0×10^{-3} M) of reagent R_3 with (1.0×10^{-3} M) of metal ions in the presence of surfactant

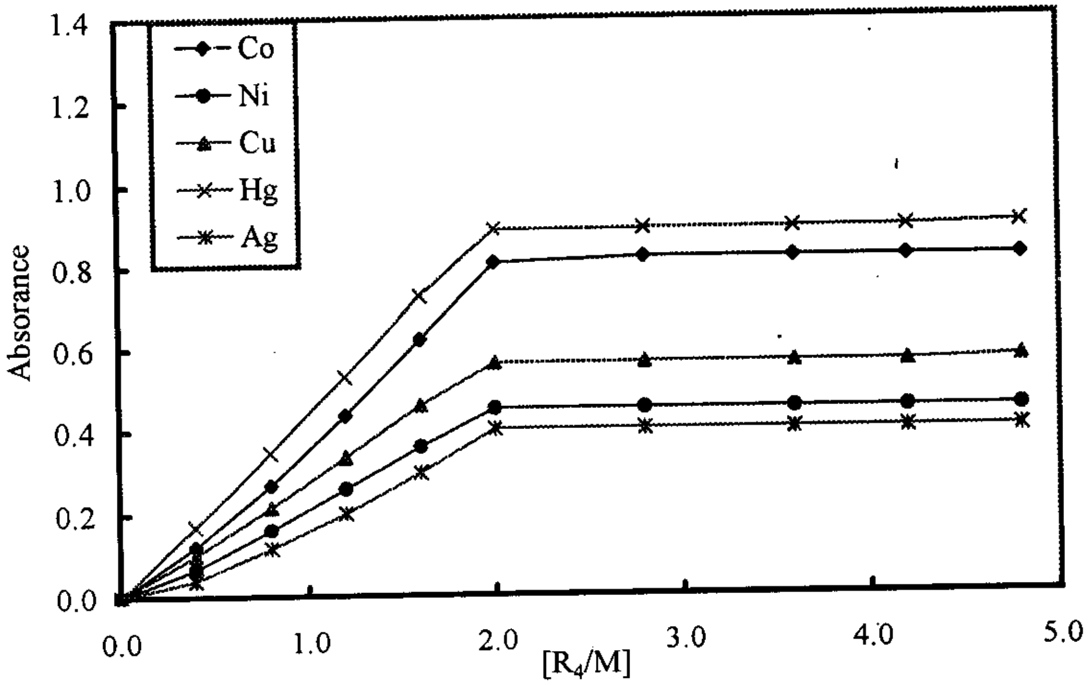


(b): Molar ratio using (1.0×10^{-3} M) of reagent R_3 with (0.5 ml of 1.0×10^{-3} M) of metal ions in the presence of surfactant

Fig. (30)

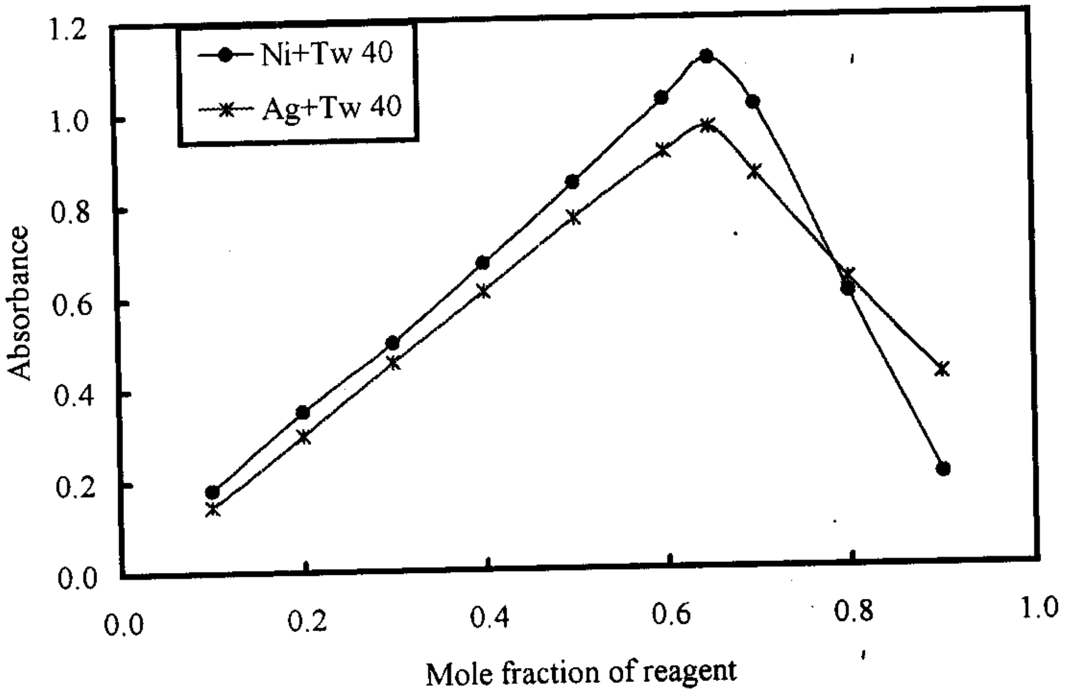


(a): Continuous variation using (1.0 x 10^{-3} M) of reagent R_4 with (1.0 x 10^{-3} M) of metal ions

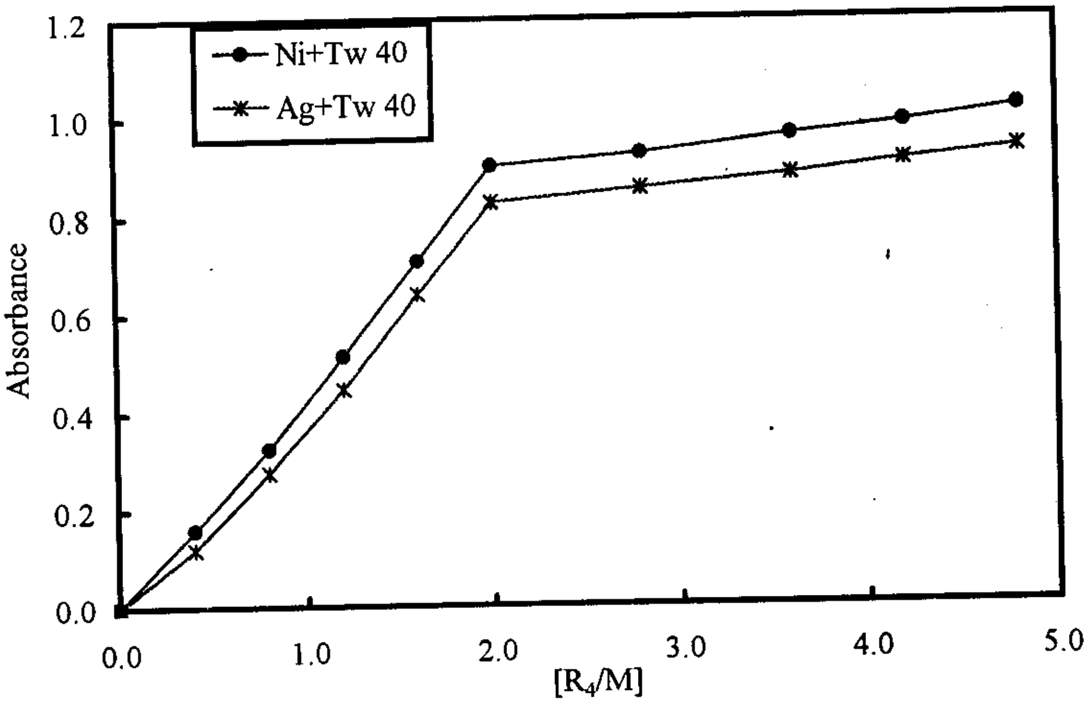


(b): Molar ratio using (1.0 x 10^{-3} M) of reagent R_4 with (0.5 ml of 1.0 x 10^{-3} M) of metal ions

Fig. (31)



(a): Continuous variation using (1.0 x 10^{-3} M) of reagent R_4 with (1.0 x 10^{-3} M) of metal ions in the presence of surfactant



(b): Molar ratio using (1.0 x 10^{-3} M) of reagent R_4 with (0.5 ml of 1.0 x 10^{-3} M) of metal ions in the presence oi surfactant

Fig. (32)

Table (15): Optimum condition parameters of reagent R_3 with the ions Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^{+}.

Parameters	**R_3**						
	Co^{2+}	**Ni^{2+}**	**Cu^{2+}**	**Hg^{2+}**		**Ag^{+}**	
				Withou^S	**With^S**	**Withou^S**	**With^S**
Working pH	10.50	9.50	5.50	5.50	5.50	9.50	9.50
λ_{max} (nm)	539	537	579	560	568	527	535
Buffer volume	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
Reagent concentration (2.0×10^{-3} M)	1.7 ml	1.7 ml	1.0 ml	1.5 ml	1.5 ml	1.5 ml	1.5 ml
Surfactant type	---	---	---	---	Tween 80	---	Tween 80
Surfactant volume	---	---	---	---	1.0 ml	---	1.0 ml
Sequence of addition	R-B-M	R-B-M	R-B-M	R-B-M	R-B-S-M	R-B-M	R-B-S-M
Stoichiometric ratio (R:M)	2:1	2:1	2:1	2:1	2:1	2:1	2:1
Stability constant^a	14.25	10.15	9.87	10.31	11.25	8.25	10.13
Stability constant^b	14.11	10.39	9.66	10.40	11.02	8.52	10.50
Average of stability constant	14.18	10.27	9.77	10.36	11.14	8.39	10.32

R: reagent B: Buffer ^{S}: surfactant M: metal

^{a}: Stability constant using molar ratio method

^{b}: Stability constant using contanous variation method

Table (16): Optimum condition parameters of reagent R_4 with the ions Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^{+}.

Parameters	**R_4**						
	Co^{2+}	**Ni^{2+}**		**Cu^{2+}**	**Hg^{2+}**	**Ag^{+}**	
		Withou^S	**With^S**			**Withou^S**	**With^S**
Working pH	10.50	9.50	9.50	5.50	5.50	9.50	9.50
λ_{max} (nm)	547	535	545	576	572	530	539
Buffer volume	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
Reagent concentration (2.0×10^{-3} M)	1.7 ml	1.7 ml	1.5 ml	1.0 ml	1.0 ml	1.5 ml	1.5 ml
Surfactant type	---	---	Tween 40	---	---	---	Tween 40
Surfactant volume	---	---	1.5 ml	---	---	---	1.5 ml
Sequence of addition	R-B-M	R-B-M	R-B-S-M	R-B-M	R-B-M	R-B-M	R-B-S-M
Stoichiometric ratio (R:M)	2:1	2:1	2:1	2:1	2:1	2:1	2:1
Stability constant[a]	10.42	10.48	11.08	10.84	11.03	9.85	10.17
Stability constant[b]	10.74	10.28	11.13	10.02	11.50	9.39	10.27
Average of stability constant	10.58	10.38	11.11	10.43	11.27	9.62	10.22

R: reagent B: Buffer ^{S}: surfactant M: metal

[a]: Stability constant using molar ratio method

[b]: Stability constant using contanous variation method

3.5.2.9. Spectrophotometric determination of metal ions

a) Validity of Beer's law

A calibration graph is constructed using standard solutions of the metal ions under cosedration in µg/ml and optimum concentration of reagent ***R₃*** or ***R₄***. Under the optimum experimental condition of pH, sequence of addition, surfactants, time and temperature a linear relationship between the absorbance and the concentration of metal ion in µg/ml is obtained upto certain concentration limit after which a deviation from ***Beer's*** law is observed as shown in Fig's. (33-36).

Experiments show that, higher molar absorptivity values are obtained and these values are increased with the addition of (1.0 ml of 0.5% v/v) of Tween 80 in case of Ag^+ and Hg^{2+} ions with reagent ***R₃***, while using (1.5 ml of 0.5% v/v) of Tween 40 leads to increase the molar absorptivity of Ni^{2+} and Ag^+ ions with reagent ***R₄***.

For more accurate results, ***Ringbom*** method is applied by plotting log [M] in µg/ml against T%. The linear portion of S-shaped curve [Fig's. 33-36] gives the accurate range of analysis. The results are recorded in Tables (17 and 18).

b) Evaluation of the accuracy and precision of the proposed method

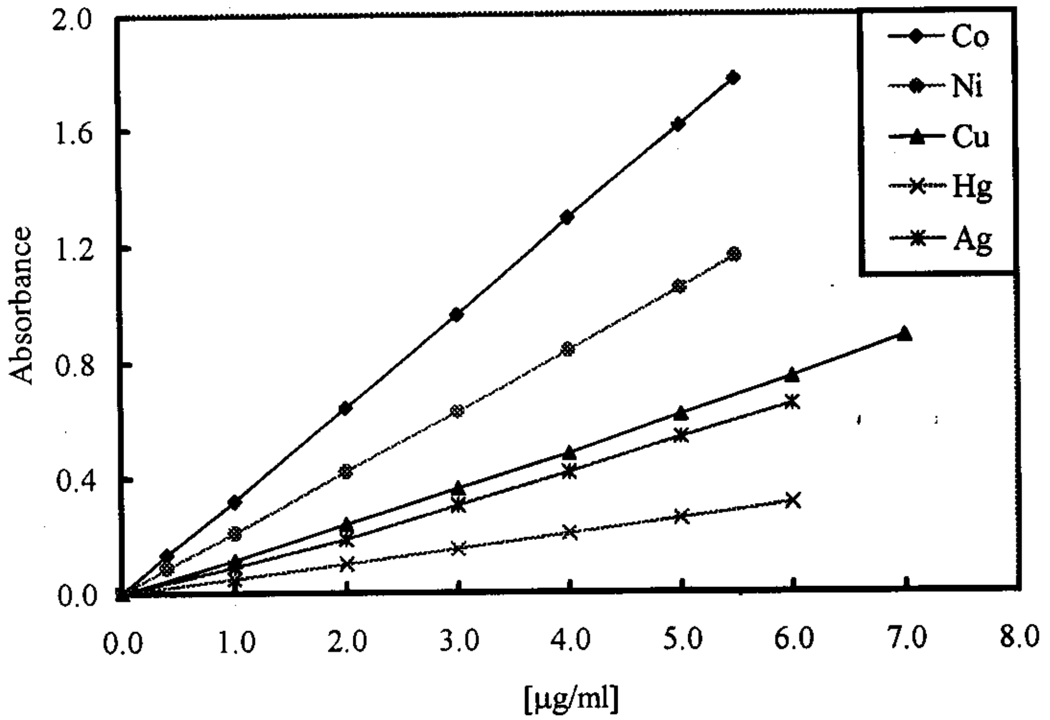
The accuracy and precision of the proposed method using reagents ***R₃*** and ***R₄*** are studied by preparing solutions containing tow different concentrations of Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^+ ions. These solutions are analysed in six replicates and the results obtained are recorded in Tables (19 and 20). The relative standard deviations, relative error and the recovery percent at 95% confidence level are calculated. The results can be considered as very satisfactory, at least for the level of concentration examined.

c) Interferences

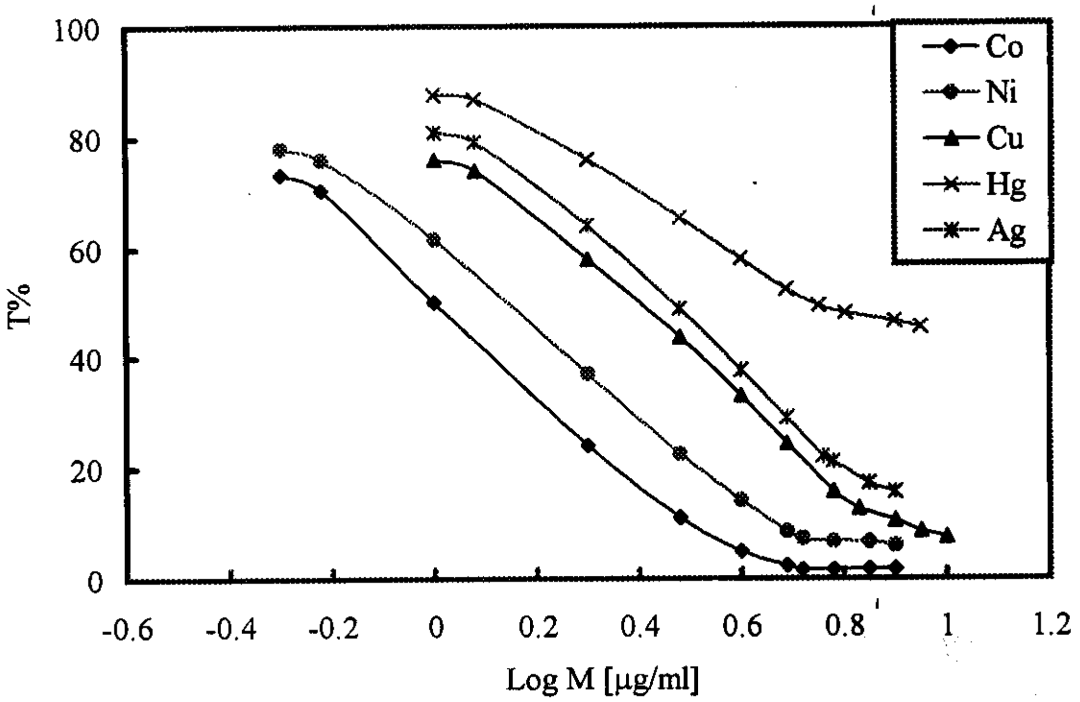
The selectivity of the present method is assessed by investigating the effect of foreign ions on the determination of metal ions under investigation. The tolerance limit is taken as the amount which caused an error of less than ±1% on each peak height. The results obtained show that anions and most inorganic metal ions like Na^+, K^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Pb^{2+}, Bi^{3+}, Al^{3+}, La^{3+}, NH_4^+, HCO_3^- SO_4^{2-}, NO^-_3, VO^-_3, Mo^{6+}, W^{6+}, oxalate, tartarate, acetate, phosphate and borate are not interfere. Whereas Zn^{2+}, Cd^{2+} and Fe^{3+} ions are interfered, so it must be removed or masked.

3.5.2.10. Analytical applications

To validate the proposed method, it is applied to determine Co^{2+} in cyanocobalamine injection, Ni^{2+}, Cu^{2+} and Ag^+ in alloy samples and Hg^{2+} in waste water. The analytical results obtained as recorded in Tables (21 and 22) shows that the proposed method is in good agreement with those obtained by AAS method with excellent (F and t-test values). The results obtained show that no signficanct difference between the proposed method and the official method indicating that the proposed method is highly sensitive during the microdetermination of Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^+ concentrations in the above real samples.

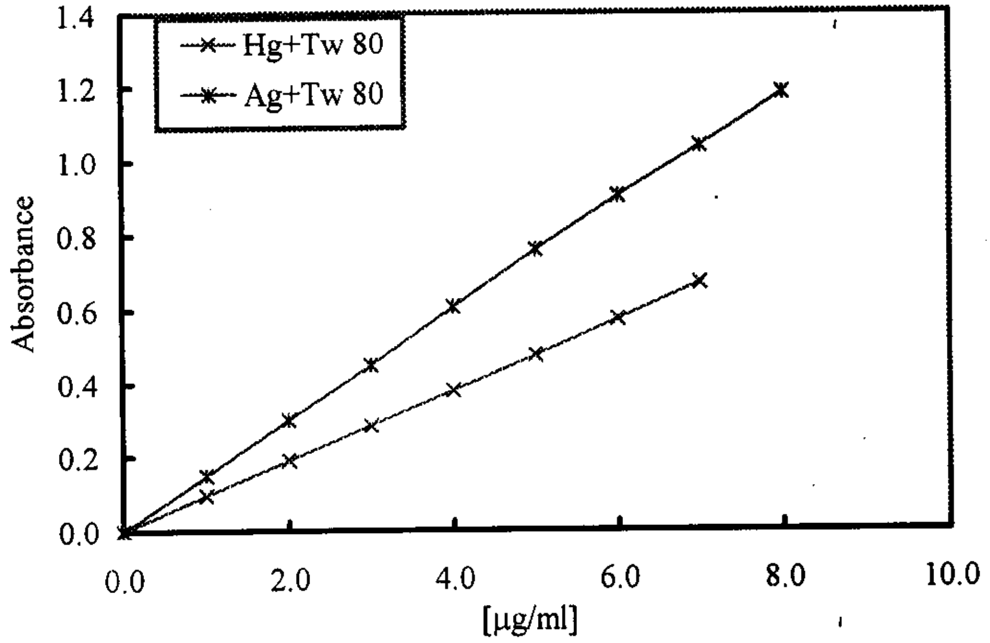


(a): Application of Beer's law for the studied metal ions complexes using the optimum volume (2.0×10^{-3} M) of reagent R_3

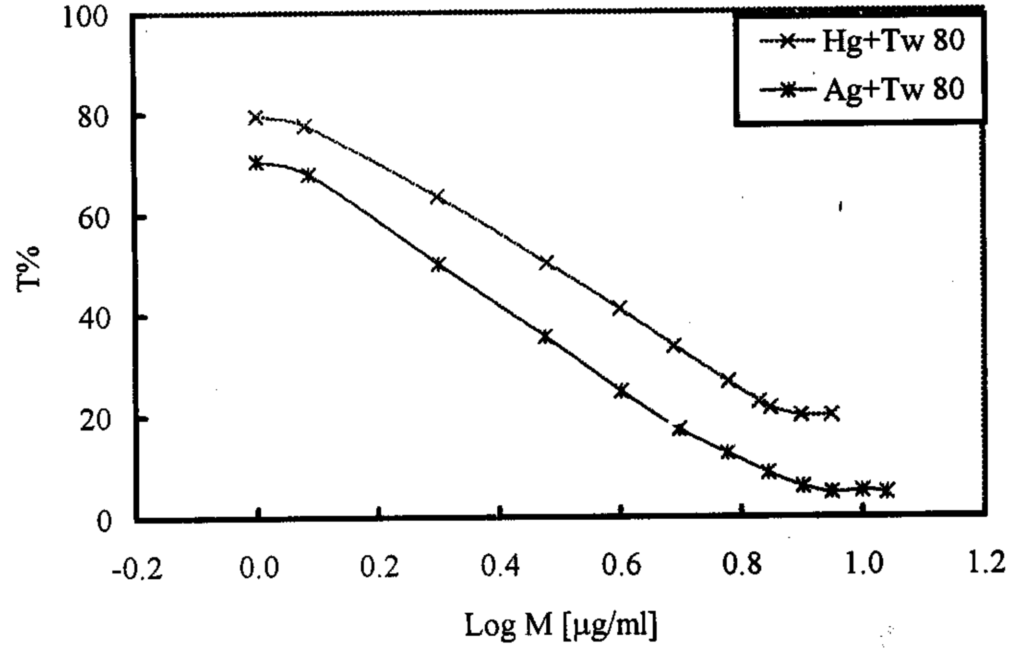


(b): Ringbom plots for the studied metal ions complexes using reagent R_3

Fig. (33)

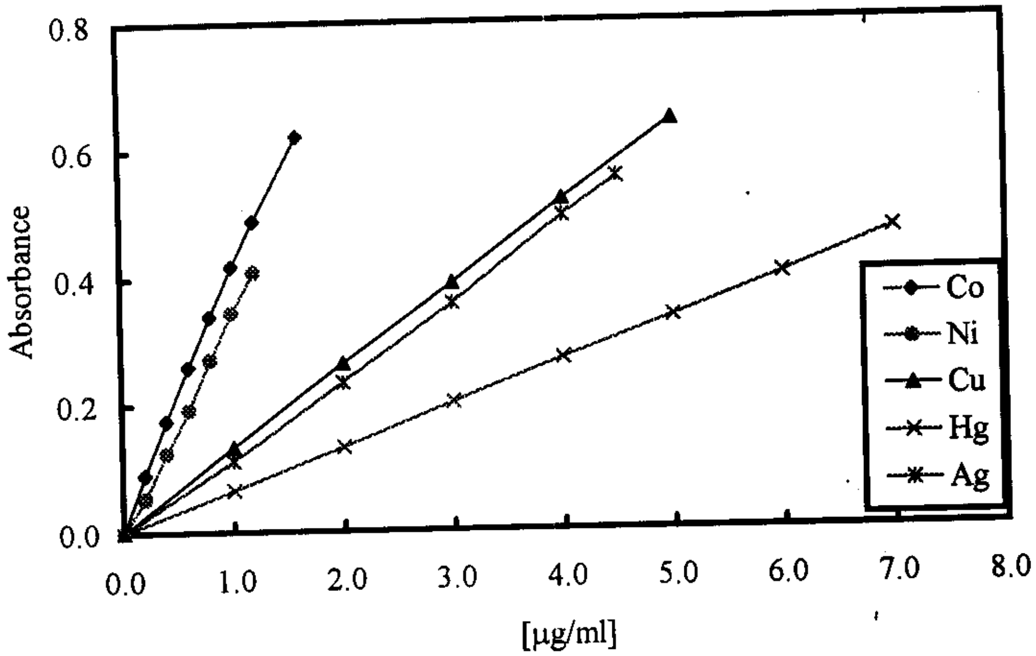


(a): Application of Beer's law for the studied metal ions complexes using the optimum volume (2.0×10^{-3} M) of reagent R_3 in the presence of surfactant

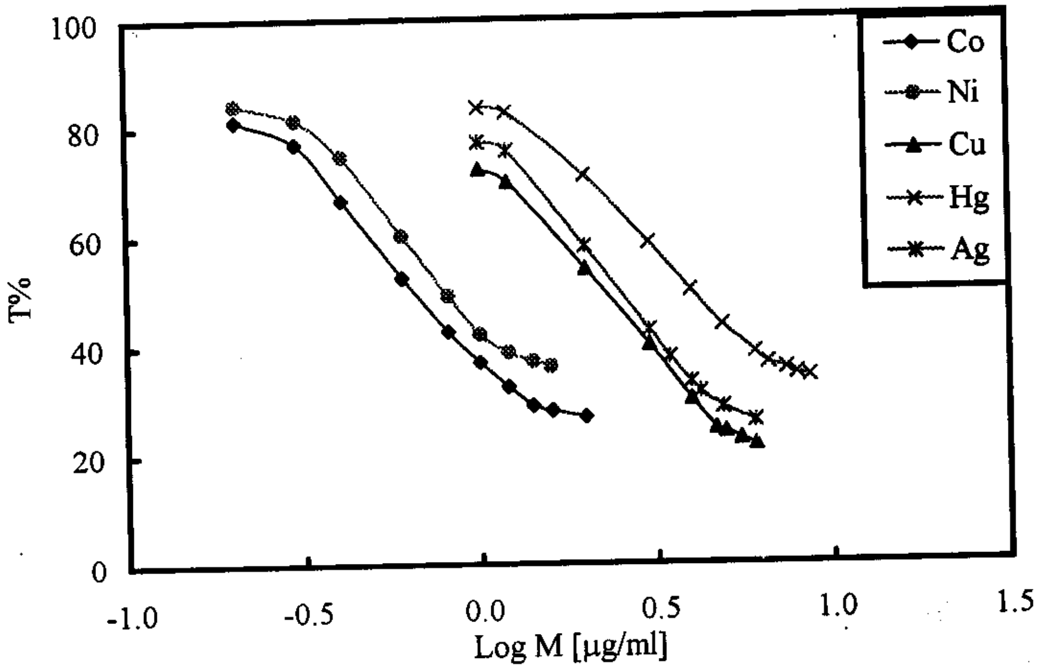


(b): Ringbom plots for the studied metal ions complexes using reagent R_3 in the presence of surfactant

Fig. (34)

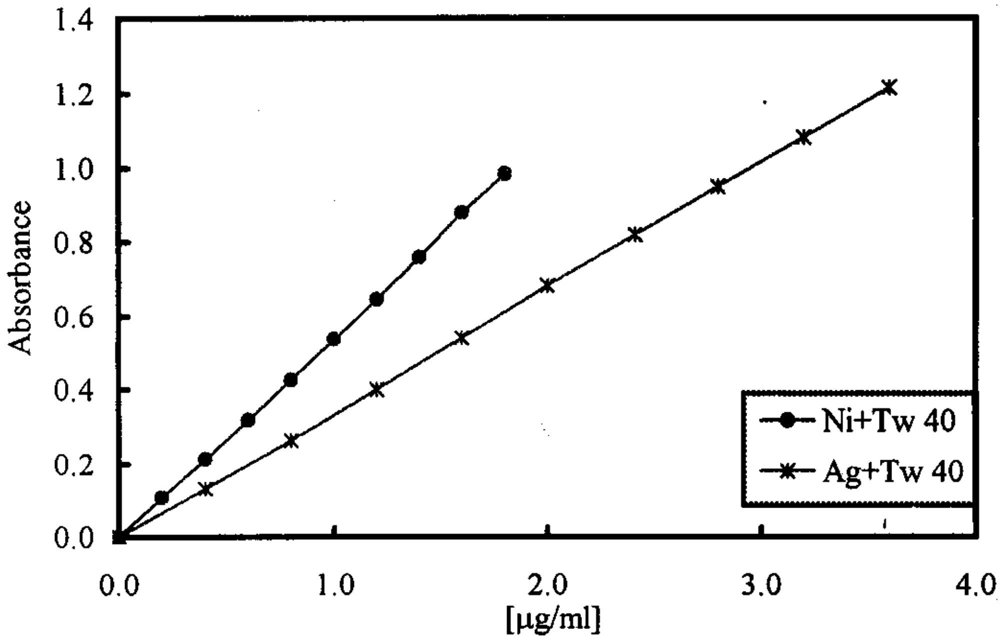


(a): Aplication of Beer's law for the studied metal ions complexes using the optimum volume (2.0×10^{-3} M) of reagent R_4

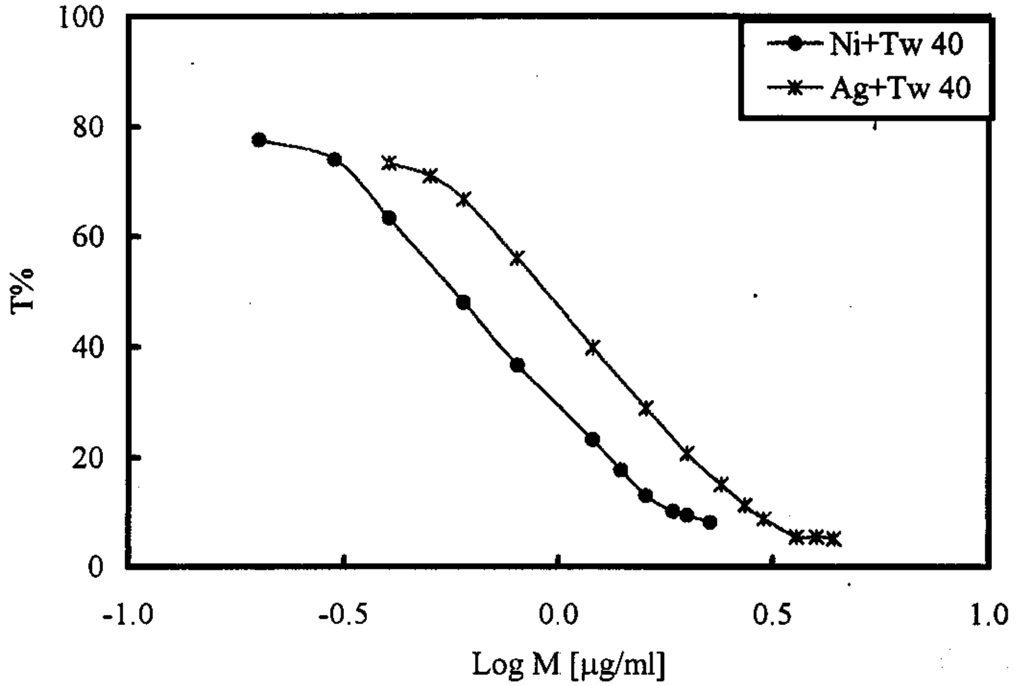


(b): Ringbom plots for the studied metal ions complexes using reagent R_4

Fig. (35)



(a): Application of Beer's law for the studied metal ions complexes using the optimum volume (2.0×10^{-3} M) of reagent R_4 in the presence of surfactant



(b): Ringbom plots for the studied metal ions complexes using reagent R_4 in the presence surfactant

Fig. (36)

Table (17): Analytical parameters of reagent R_3 complexes with Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^{+} ions.

Parameters	**R_3**						
	Co^{2+}	**Ni^{2+}**	**Cu^{2+}**	**Hg^{2+}**		**Ag^{+}**	
				Withou^{S}	**With^{S}**	**Withou^{S}**	**With^{S}**
pH	10.50	9.50	5.50	5.50	5.50	9.50	9.50
λ_{max} (nm)	539	537	579	560	568	527	535
Beer's range (μg/ml)	0.5-5.5	0.5-5.5	1.0-7.0	1.0-6.0	1.0-7.0	1.0-6.0	1.0-8.0
Ringbom range (μg/ml)	0.6-5.3	0.6-5.2	1.2-6.7	1.2-5.7	1.2-6.8	1.2-5.8	1.2-7.7
Detection Limit (μg/ml)	0.089	0.113	0.153	0.279	0.113	0.105	0.134
Quantification limit (μg/ml)	0.299	0.378	0.508	0.932	0.376	0.349	0.446
SD*	0.0078	0.0061	0.0067	0.0072	0.0069	0.0077	0.0093
RSD %	0.816	0.66	0.235	0.278	0.302	0.463	0.612
Error %	0.326	0.245	0.286	0.286	0.245	0.286	0.367
Slope	0.319	0.213	0.121	0.052	0.096	0.109	0.150
Intercept	-0.004	-0.002	-0.001	-0.003	-0.003	-0.001	-0.004
Correlation Coefficient	0.9998	0.9999	0.9998	0.9997	0.9998	0.9997	0.9999
Molar absorptivity x 10^4 (l. mol^{-1} cm^{-1})	1.88	1.25	0.77	1.04	1.92	1.18	1.62
Sandell Sensitivity (ng cm^{-2})	3.13	4.69	8.26	19.23	10.42	9.17	6.67

*: Average of six determinations

^{S}: surfactant

Table (18): Analytical parameters of reagent R_4 complexes with Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^{+} ions.

Parameters	**R_4**						
	Co^{2+}	**Ni^{2+}**		**Cu^{2+}**	**Hg^{2+}**	**Ag^{+}**	
		Withou^S	**With^S**			**Withou^S**	**With^S**
pH	10.50	9.50	9.50	5.50	5.50	9.50	9.50
λ_{max} (nm)	547	535	543	576	572	530	538
Beer's range (μg/ml)	0.2-1.6	0.2-1.2	0.2-1.8	1.0-5.0	1.0-7.0	1.0-4.5	0.4-3.6
Rangbom range (μg/ml)	0.3-1.4	0.3-1.0	0.3-1.6	1.2-4.8	1.2-6.8	1.2-4.2	0.5-3.4
Detection Limit (μg/ml)	0.038	0.064	0.046	0.276	0.291	0.297	0.073
Quantification limit (μg/ml)	0.128	0.212	0.152	0.922	0.969	0.822	0.244
SD*	0.0081	0.0064	0.0078	0.0089	0.0092	0.0081	0.0094
RSD %	0.567	1.170	1.011	0.833	0.569	0.396	0.608
Error %	0.327	0.245	0.327	0.367	0.367	0.327	0.367
Slope	0.403	0.348	0.546	0.129	0.067	0.124	0.340
Intercept	0.005	-0.003	-0.005	-0.001	-0.003	-0.003	-0.001
Correlation Coefficient	0.9998	0.9998	0.9998	0.9999	0.9998	0.9997	0.9998
Molar absorptivity x 10^4 ($l.\ mol^{-1}\ cm^{-1}$)	2.37	2.04	3.20	0.82	1.34	1.34	3.67
Sandell Sensitivity (ng cm^{-2})	2.48	2.87	1.83	7.75	14.93	8.06	2.94

*: Average of six determinations

^{S}: surfactant

Table (19): Evaluation of the accuracy and precision of the proposed method using reagent R_3.

Sample	Taken (μg/ml)	Found[*] (μg/ml)	Recovery %	RSD %	Relative Error	Confidence limits[**]
$CoCl_2$	2.0	1.999	99.95	0.225	-0.05	1.999 ± 0.0047
	4.0	4.032	100.80	0.238	+0.20	4.032 ± 0.0101
$NiCl_2$	2.0	2.028	101.40	0.404	+1.40	2.028 ± 0.0086
	4.0	3.991	99.77	0.235	-0.33	3.991 ± 0.0099
$CuCl_2$	2.0	2.017	100.85	0.376	+0.85	2.017 ± 0.0079
	4.0	3.997	99.93	0.217	-0.07	3.997 ± 0.0091
$AgNO_3$	2.0	2.025	101.25	0.469	+1.25	2.025 ± 0.0093
		2.012^{S}	100.60^{S}	0.442^{S}	$+1.60^{S}$	2.012 ± 0.0091^{S}
	4.0	3.988	99.70	0.198	-0.30	3.988 ± 0.0083
		4.025^{S}	100.63^{S}	0.238^{S}	$+1.63^{S}$	4.025 ± 0.1001^{S}
$HgCl_2$	2.0	1.997	99.85	0.495	-0.15	1.997 ± 0.0104
		2.019^{S}	100.95^{S}	0.302^{S}	-0.05^{S}	2.019 ± 0.0062^{S}
	4.0	4.011	100.27	0.209	+0.27	4.011 ± 0.0088
		4.013^{S}	100.33^{S}	0.184^{S}	$+0.33^{S}$	4.013 ± 0.0077^{S}

[*]: Average of six determinations.

[**]: 95% confidence limits and five degrees of freedom.

s: the calculated values in the presence of surfactants.

Table (20): Evaluation of the accuracy and precision of the proposed method using reagent R_4.

Sample	Taken (μg/ml)	Found* (μg/ml)	Recovery %	RSD %	Relative Error	Confidence limits**
$CoCl_2$	0.5	0.499	99.80	1.022	-0.20	0.499 ± 0.0054
	1.0	1.008	100.80	0.912	+0.80	1.008 ± 0.0097
$NiCl_2$	0.5	0.502	100.40	1.075	+0.40	0.502 ± 0.0057
		0.505^{S}	101.00^{S}	0.970^{S}	$+1.00^{S}$	0.505 ± 0.0051^{S}
	1.0	0.997	99.70	0.872	+0.30	0.997 ± 0.0091
		1.012^{S}	101.20^{S}	0.662^{S}	$+1.20^{S}$	1.012 ± 0.0070^{S}
$CuCl_2$	2.0	1.998	99.90	0.375	-0.10	1.998 ± 0.0067
	4.0	3.997	99.93	0.247	-0.07	3.977 ± 0.0104
$AgNO_3$	1.0	1.011	101.10	0.682	+1.10	1.011 ± 0.0072
		0.998^{S}	99.80^{S}	0.942^{S}	-0.20^{S}	0.998 ± 0.0098^{S}
	3.0	2.996	99.86	0.303	-0.14	2.996 ± 0.0096
		2.998^{S}	99.93^{S}	0.287^{S}	-0.07^{S}	2.998 ± 0.0090^{S}
$HgCl_2$	2.0	2.018	100.90	0.401	+0.90	2.018 ± 0.0085
	4.0	4.029	100.73	0.193	+0.73	4.029 ± 0.0082

*: Relative standard deviation for six determinations.

**: 95% confidence limits and five degrees of freedom.

s: the calculated values in the presence of surfactants.

Table (21): Evaluation of the accuracy and precision of the proposed method for determination of metal ions in real samples using reagent R_3..

Sample	Metal %	Metal Found (%)					
		AAS. method		Proposed method			
		Found* %	Recovery %	Found* %	Recovery %	t$^{\#}$ test	F$^{\#}$ value
Vitamine B_{12}	0.05	0.0502	100.40	0.0499	99.80	1.77	3.14
Ni^{2+} in (Ni-Cu alloy)	50	50.101	100.20	49.992	99.96	1.98	3.53
Cu^{2+} in (Ni-Cu alloy)	50	50.302	100.60	50.124	100.24	1.31	2.25
Cu^{2+} in (Ag-Cu alloy)	20	19.962	99.81	20.091	100.45	1.36	2.92
Ag^{2+} in (Ag-Cu alloy)	80	79.983	99.98	80.611	100.76	1.72	3.16
				80.149	100.19^{S}	1.43^{S}	2.84^{S}
Hg^{2+} in waste water	0.156	0.157	100.64	0.1562	100.13	1.69	3.25
				0.1564^{S}	100.26^{S}	1.54^{S}	3.12^{S}

*: Average of six determinations.

$^{\#}$: Theoretical values for t-and F- values for five dgree of freedom and 95% confidence limits are 2.57 and 5.05, respectively.

^{S}: The calculated values in the presence of surfactants.

Table (22): Evaluation of the accuracy and precision of the proposed method for determination of metal ions in real samples using reagent R_4.

Sample	Metal %	Metal Found (%)					
		AAS. method		Proposed method			
		Found* %	Recovery %	Found* %	Recovery %	t* test	F# value
Vitamine B_{12}	0.05	0.0502	100.40	0.0502	100.40	0.78	2.43
Ni^{+} in (Ni-Cu alloy)	50	50.101	100.20	50.256	100.50	1.48	3.65
				50.321^{S}	100.64^{S}	0.94^{S}	3.23^{S}
Cu^{2+} in (Ni-Cu alloy)	50	50.302	100.60	50.422	100.84	1.82	3.80
Cu^{2+} in (Ag-Cu alloy)	20	19.962	99.81	20.147	100.70	0.84	3.36
Ag^{2+} in (Ag-Cu alloy)	80	79.983	99.98	79.971	99.96	0.54	2.62
				79.942^{S}	99.93^{S}	0.63^{S}	2.98^{S}
Hg^{2+} in waste water	0.156	0.157	100.64	0.1561	100.60	0.68	2.21

: Average of six determinations.

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

S: The calculated values in the presence of surfactants.

Table (23): Determination of the studied metal ions in real samples applying the standard addition techinque using reagent R_3.

Sample	Taken (µg/ml)	Added (µg/ml)	Found* (µg/ml)		Recovery %	
Co^{2+} in (vitamine B_{12})	3.0	--	3.01		100.33	
		1.0	3.98		99.50	
		1.5	4.51		100.22	
		2.0	5.04		100.80	
Ni^{2+} in (Ni-Cu alloy)	3.0	--	2.97		99.00	
		1.0	4.03		100.75	
		1.5	4.55		101.11	
		2.0	4.98		99.60	
Cu^{2+} in (Ni-Cu allow)	3.0	--	2.98		99.33	
		1.0	3.98		99.50	
		2.0	5.03		100.60	
		3.0	6.01		100.17	
Cu^{2+} in (Ag-Cu alloy)	3.0	--	2.98		99.33	
		1.0	4.04		101.00	
		2.0	4.97		99.40	
		3.0	6.03		100.50	
Ag^+ in (Ag-Cu alloy)	3.0	--	3.03	2.99^S	101.00	99.67^S
		1.0	4.04	4.02^S	100.10	101.00^S
		2.0	4.98	5.01^S	99.60	100.20^S
		3.0	6.01	6.03^S	100.17	100.50^S
Hg^{2+} in waste water	3.0	--	3.02	3.01^S	100.67	100.33^S
		1.0	4.01	3.99^S	100.25	99.75^S
		2.0	5.02	4.98^S	100.40	99.60^S
		3.0	5.97	6.02^S	99.50	100.33^S

*: Average of six determinations

^{S}: The calculated values in the presence of surfactant

Table (24): Determination of the studied metal ions in real samples applying the standard addition techinque using reagent R_4.

Sample	Taken (µg/ml)	Added (µg/ml)	Found* (µg/ml)		Recovery %	
Co^{2+} in (vitamine B_{12})	0.4	--	0.41		102.50	
		0.4	0.79		98.75	
		0.6	0.98		98.00	
		0.8	1.21		100.83	
Ni^{2+} in (Ni-Cu alloy)	0.4	--	0.39	0.40^{S}	97.50	100.00^{S}
		0.4	0.81	0.79^{S}	101.25	98.75^{S}
		0.6	1.01	1.02^{S}	101.00	102.00^{S}
		0.8	1.18	1.21^{S}	98.33	100.83^{S}
Cu^{2+} in (Ni-Cu alloy)	2.5	--	2.51		100.40	
		1.0	3.48		99.43	
		1.5	3.97		99.25	
		2.0	4.54		100.88	
Cu^{2+} in (Ag-Cu alloy)	2.5	--	2.51		100.40	
		1.0	3.51		100.29	
		1.5	4.02		100.50	
		2.0	4.48		99.55	
Ag^{+} in (Ag-Cu alloy)	1.0	--	0.99	1.01^{S}	99.00	101.00^{S}
		0.5	1.48	1.52^{S}	98.66	101.33^{S}
		1.0	2.02	2.01^{S}	101.00	100.50^{S}
		2.0	3.03	3.01^{S}	100.67	100.33^{S}
Hg^{2+} in waste water	3.0	--	2.98		99.33	
		1.0	4.02		100.50	
		2.0	4.98		99.60	
		3.0	6.02		100.33	

*: Average of six determinations

S: The calculated values in the presence of surfactant

3.5.3. Spectrophotometric studies of [2-amino-4-(p-tolylazo) pyridine-3-ol] R_5, [2-amino-4-(3-nitrophenylazo) pyridine-3-ol] R_6 complexes with Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^{+} ions

In order to investigate the optimum conditions for complexation of the metal ions Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^{+} with the reagents R_5 and R_6, the effect of different experimental variables are extensively studied as recorded below.

3.5.3.1. Effect of pH

In a trial to elucidate the optimum buffer medium for maximum formation of complexes between reagents R_5 and R_6 with Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^{+} ions in universal buffer solutions. The studies are carried out in solution of different pH values (2.50-11.50).The higher absorbance values of the complexes formed with R_5 are obtained in buffer medium of (pH 9.50-10.50) as show in Fig. (37). On using R_6 the higher absorbance values are found at (pH 8.0-8.50) as show in Fig. (38). Also the optimum volume of buffer solution required for complete complexation is examined and the results show that 2.0 ml from the selected buffer solution is sufficient for reagent-metal complex formation.

3.5.3.2. Determination of maximum wavelength of complex species

The maximum wavelength at which the metal ions complexes are formed is investigated. Representative curves for Cu^{2+} and Ag^{+} complexes with R_5 and R_6, respectively, are showed in Fig. (39). The results show that reagent R_5 and Cu^{2+} complex are absorbed at 468 and 495 nm, respectively, with buffer as a blank (curve A and B). When reagent R_5 used as a blank, Cu^{2+} complex is absorbed at 536 nm (curve C). The addition of 1.5 ml of

0.5% (v/v) of Tween 80 increases the absorbance and shifts the λ_{max} of Cu^{2+} complex from 536 to 543 nm (curve D). Also experiments show that the absorbance of Ag^{+} complex is increass and its λ_{max} is shifted from 523 to 531 nm with the addition of 1.5 ml of 0.5% (v/v) of Tween 40. Whereas the absorbance and the λ_{max} of Co^{2+}, Ni^{2+} and Hg^{2+} complexes with $\boldsymbol{R_5}$ are not affected with the presence of surfactants. The maximum wavelengths of metal complexes are recorded in Table (25).

For reagent $\boldsymbol{R_6}$ it is found that, the reagent and Ag^{+} complex are absorbed maximally at 480 and 490 nm, respectively, with buffer as a blank (curve A and B). In using reagent $\boldsymbol{R_6}$ and buffer as a blank, Ag^{+} complex is absorbed at 546 nm (curve C). The addition of 2.0 ml of 0.5% Tween 80 increases the absorbance and shifts the λ_{max} of Ag^{+} complex from 546 to 556 nm (curve D). Also the results show that the addition of 1.5 ml of 0.5% (v/v) Tween 80 increases the absorbance of Hg^{2+} complex and does not change in its λ_{max}. The maximum wavelength of Co^{2+}, Ni^{2+} and Cu^{2+} complexes with $\boldsymbol{R_6}$ is recorded in Table (26).

3.5.3.3. Effect of surfactants

Since the surfactants are used to improve the sensitivity of the resulted complexes, experiments on using surfactants show that the addition of 1.5 ml of 0.5% (v/v) Tween 80 and Tween 40 for Cu^{2+} and Ag^{+} complexes, respectively, with $\boldsymbol{R_5}$ increases the absorbance and red shifts their λ_{max} by 7.0 and 8.0 nm, respectively. The other metals show weak response for the presence of surfactants. On using $\boldsymbol{R_6}$ as spectrophotometric reagent for metal ion determination, it is found that the effect of adding 1.5 ml of 0.5% (v/v) of Tween 80 increases the absorbance of Hg^{2+} complex but does not change its maximum wavelength.

In case of Ag^+ complex with the same reagent, the addition of 2.0 ml of the same surfactant leads to an increase in maximum absorbance and a red shift by about 10 nm in its λ_{max}. The other metal shows weak response for the presence of surfactants.

3.5.3.4. Effect of reagent concentration

To establish the optimum concentration of reagents R_5 and R_6, different volumes of (2.0 x 10^{-3} M) of reagent are added to 2.0 ml of the selected buffer while the concentration of metal ion is kept constant at (1.0 ml of 1.0 x 10^{-3} M). For the metal ions that have a sensitivity to the surfactants the optimum amount of surfactant is added to both sample and blank. The results obtained as shown in Fig's. (40 and 41) show that the optimum volume of (2.0 x 10^{-3} M) of R_5 are 1.7 and 1.5 ml in case of Co^{2+} and Ni^{2+} complexes respectively, and 1.0 ml from the same reagent in case of Cu^{2+}, Hg^{2+} and Ag^+.

On using R_6, it is found that 1.0 ml of (2.0 x 10^{-3} M) in case of Co^{2+}, Ni^{2+} and Hg^{2+} are enough to produce maximum absorbance value, while 1.2 and 1.5 ml from the same reagent are sufficient in case of Cu^{2+} and Ag^+ complexes respectively. The optimum concentration of reagent R_5 and R_6 in the presence of surfactant are recorded in Tables (25 and 26).

3.5.3.5. Effect of time and temperature

Experiments show that the complexes are formed after mixing the reagent and metal and remain constant for at least 24 h except Ni^{2+} and Co^{2+} complexes with R_6 that show high absorbance after 5.0 min. Also no change in the absorbance occurrs on increasing the temperature up to 60 °C, above which the absorbance is fade slowly.

3.5.3.6. Effect of sequence of addition

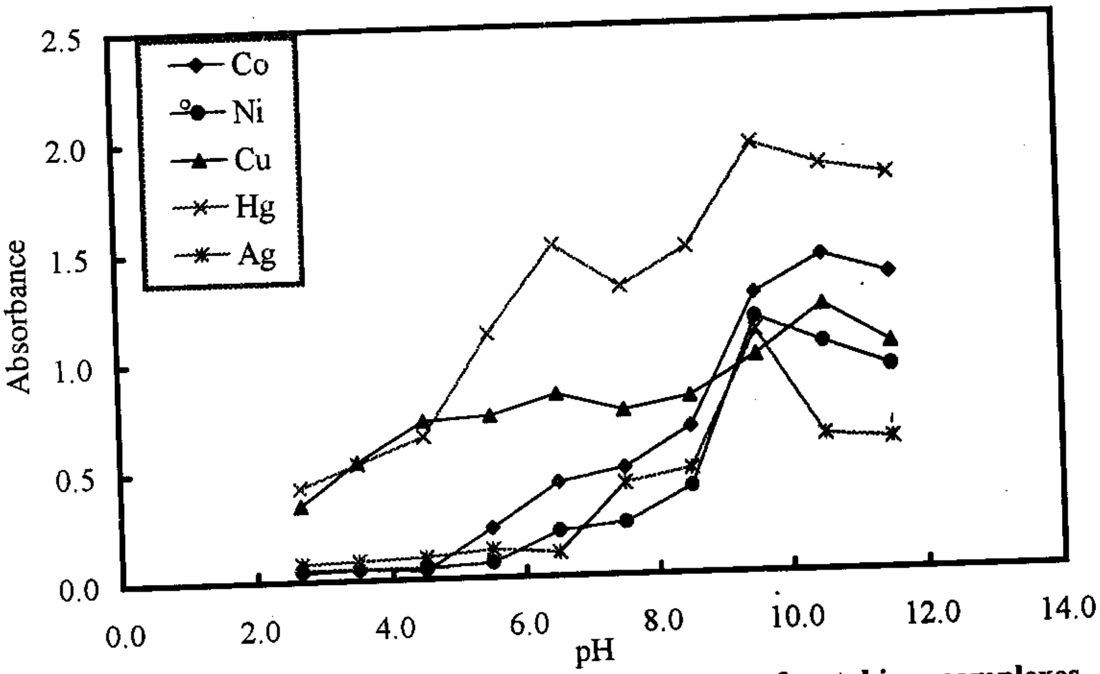
Experiments show that the order (reagent-buffer-surfactant-metal) gives the best results for Cu^{2+} and Ag^{+} complexes with reagent $\boldsymbol{R_5}$ and Hg^{2+} and Ag^{+} complexes with reagent $\boldsymbol{R_6}$ in the presence of Tween 40 and Tween 80. The order (reagent-buffer-metal) is favoured for all metal ions complexes with the same reagents in the absence of surfactants. Other sequences give lower absorbance values under the same conditions.

3.5.3.7. Molecular structure of metal complexes

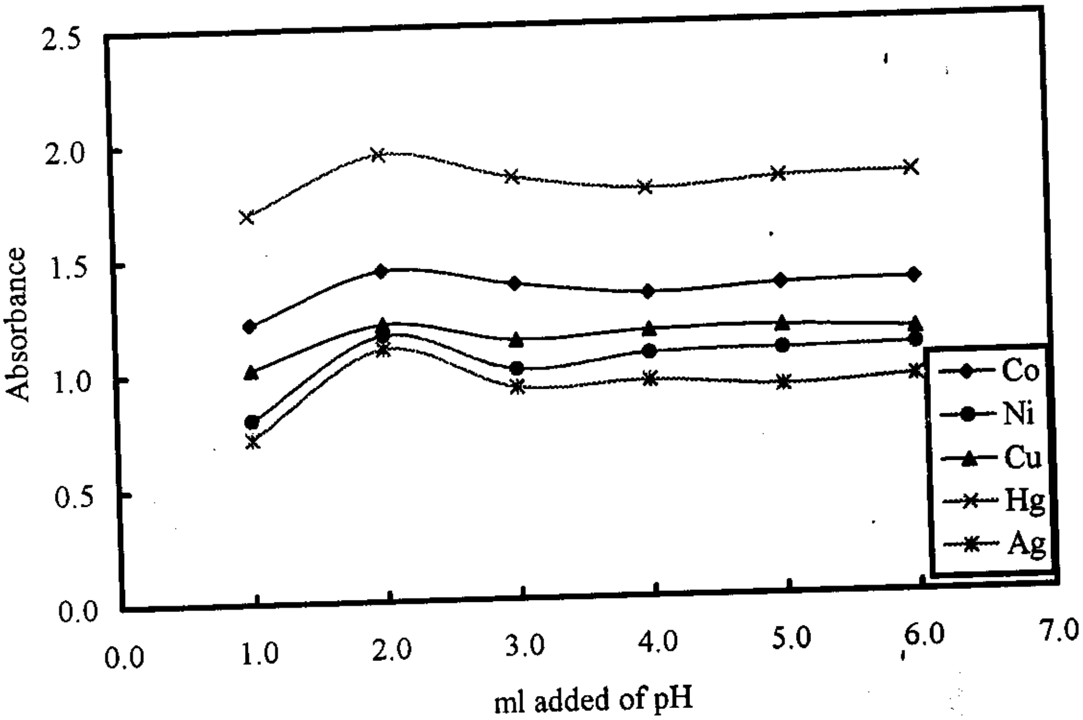
Investigation of the mole ratio of the complexes that are formed between the metal ions Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^{+} with reagents $\boldsymbol{R_5}$ and $\boldsymbol{R_6}$ at the selected conditions is described by using the molar ratio and continuous variation methods. The results obtained indicates that the mole ratio of reagents $\boldsymbol{R_5}$ and $\boldsymbol{R_6}$ to metal ions is found to be 2:1 (R:M) in all metal ion complexes as shown in Fig's. (42-45).

3.5.3.8. Stability constant of the formed complexes

The stability constant of the metal ion complexes are calculated by using the data of molar ratio and continuous variation methods applying ***Issa*** method. The careful investigation of the results obtained shows that the metal complexes with $\boldsymbol{R_5}$ and $\boldsymbol{R_6}$ have higher stability constant values and these values are increased in case of Cu^{2+} and Ag^{+} complexes with $\boldsymbol{R_5}$ with the addition of 1.5 ml of 0.5% Tween 80 and Tween 40, respectively, while Hg^{2+} and Ag^{+} complexes with $\boldsymbol{R_6}$ show higher stability constant with the addition of 1.5 ml of 0.5% Tween 80 in comparsion with the same metal ions without surfactants. The results are listed in Tables (25 and 26).

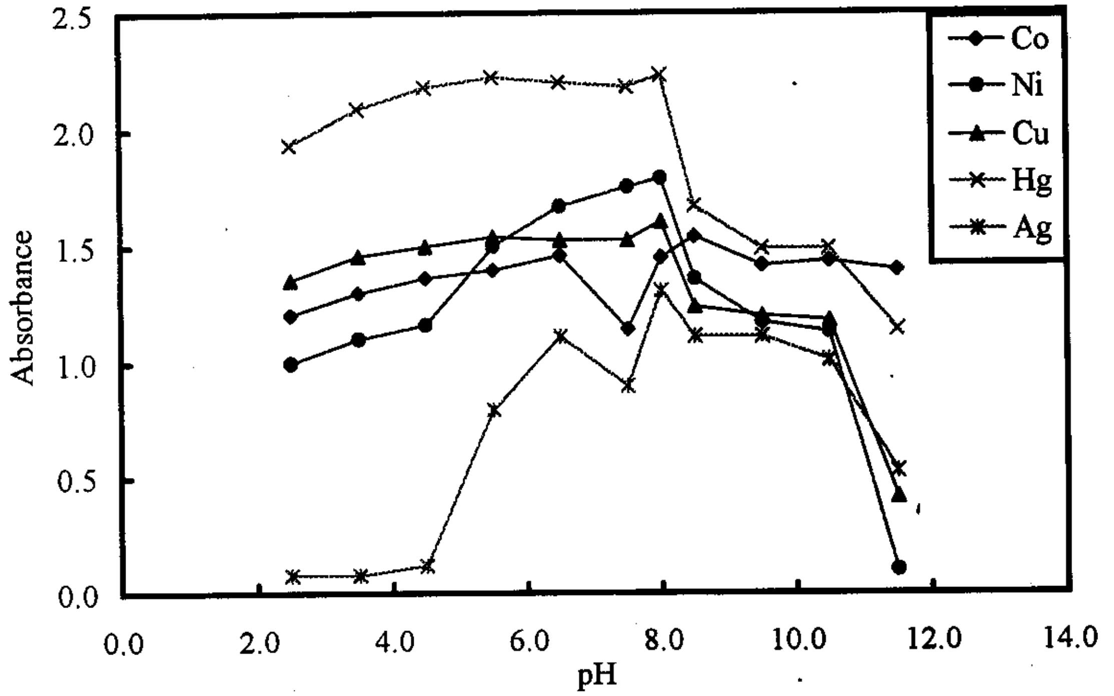


(a): Effect of pH on the maximum absorbances of metal ions complexes using (2.0 x 10^{-3} M) of reagent R$_5$

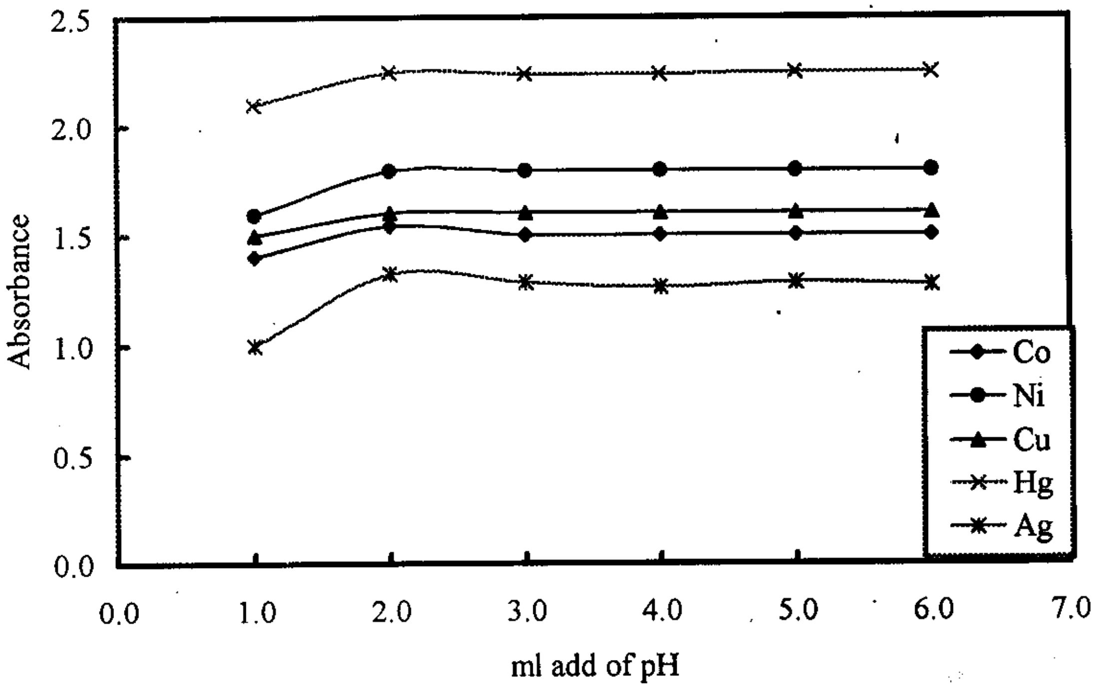


(b): Effect of ml added of buffer solution of the recommended pH on the complex formation using (2.0 x 10-3 M) of reagent R$_5$

Fig. (37)

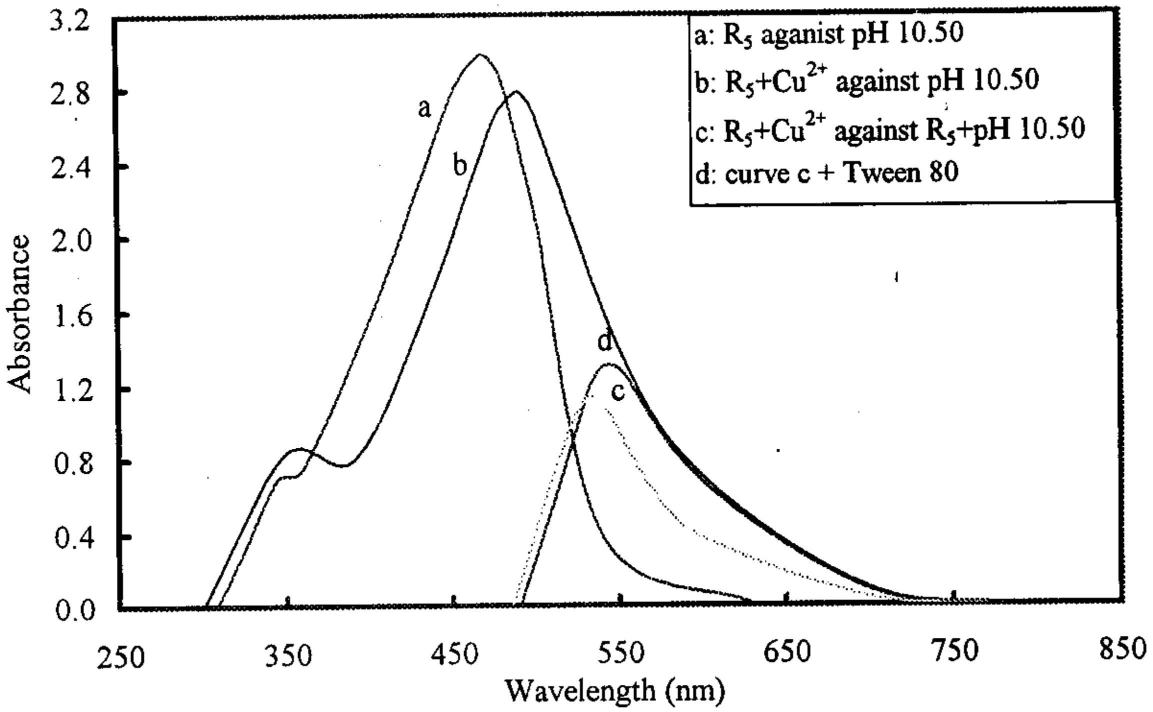


(a): Effect of pH on the maximum absorbances of metal ions complexes using (2.0×10^{-3} M) of reagent R_6

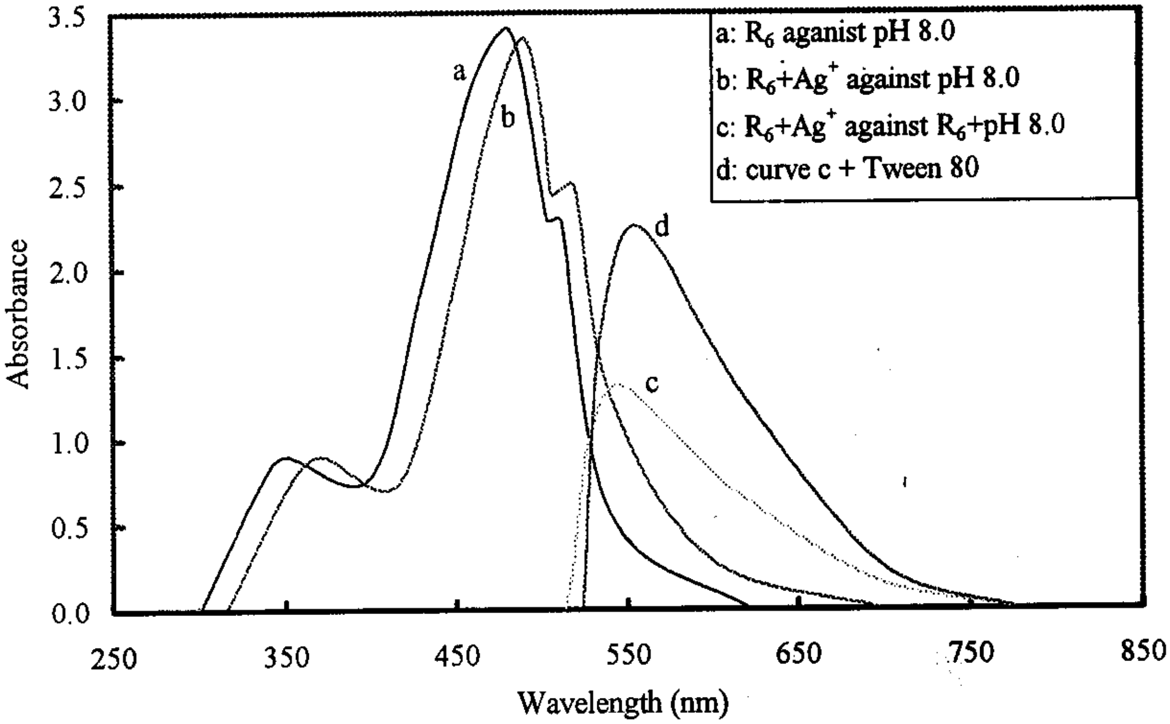


(b): Effect of ml added of buffer solution of the recommended pH on the complex formation using (2.0×10^{-3} M) of reagent R_6

Fig. (38)

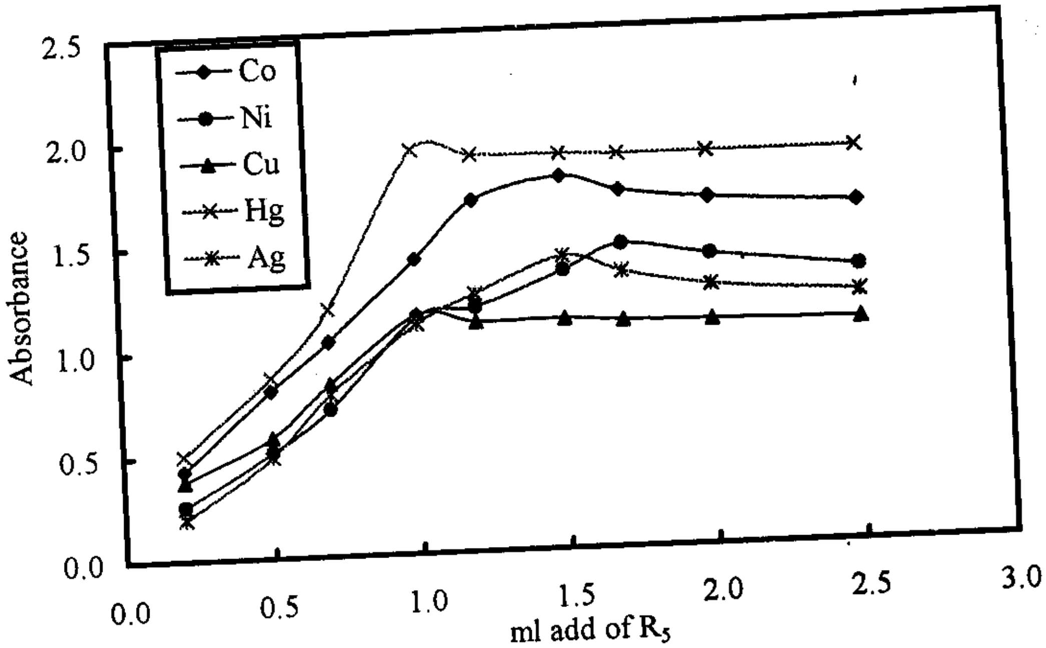


(a): Absorption spectra of (2 .0 x 10^{-3} M) R_5 with (1.0 x 10^{-3} M) of Cu^{2+} in the presence of (1.5 ml 0.5% v/v) Tween 80

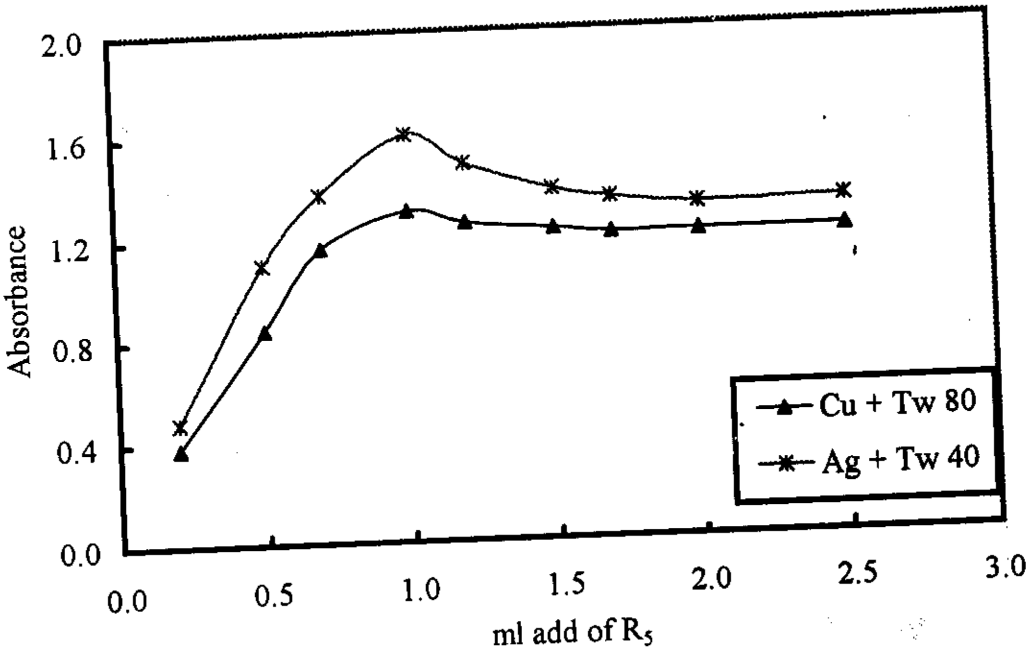


(b): Absorption spectra of (2 .0 x 10^{-3} M) R_6 with (1.0 x 10^{-3} M) of Ag^+ in the presence of (1.5 ml 0.5% v/v) Tween 80

Fig. (39)

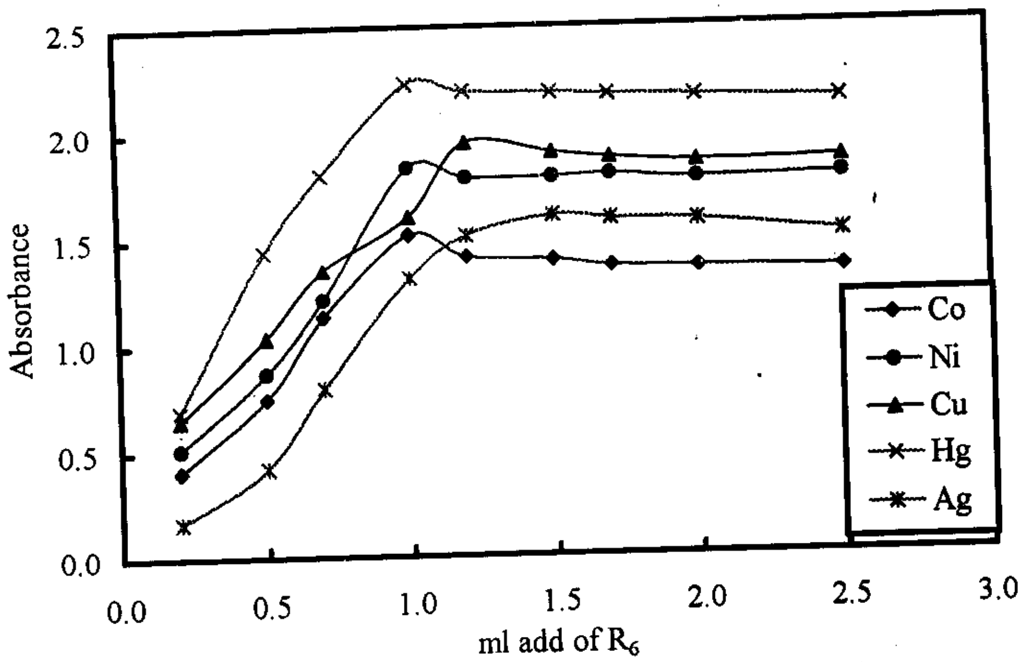


(a): Effect of reagent concentration on the absorbance of metal ions complexes using (2.0×10^{-3} M) of reagent R_5

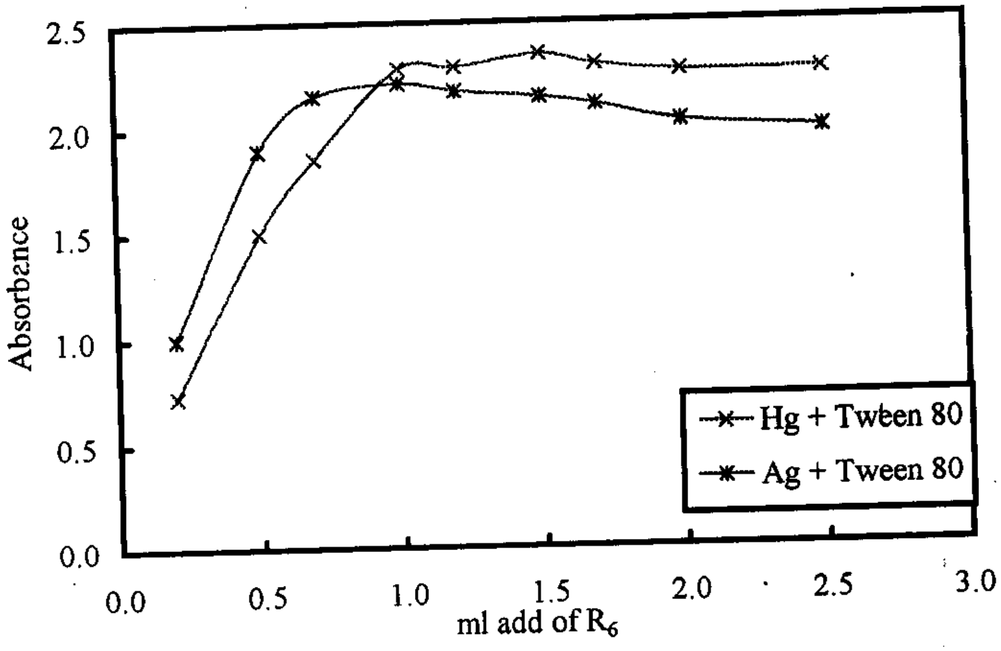


(b): Effect of reagent concentration on the absorbance of metal ions complexes in the presence of surfactants using (2.0×10^{-3} M) of reagent R_5

Fig. (40)

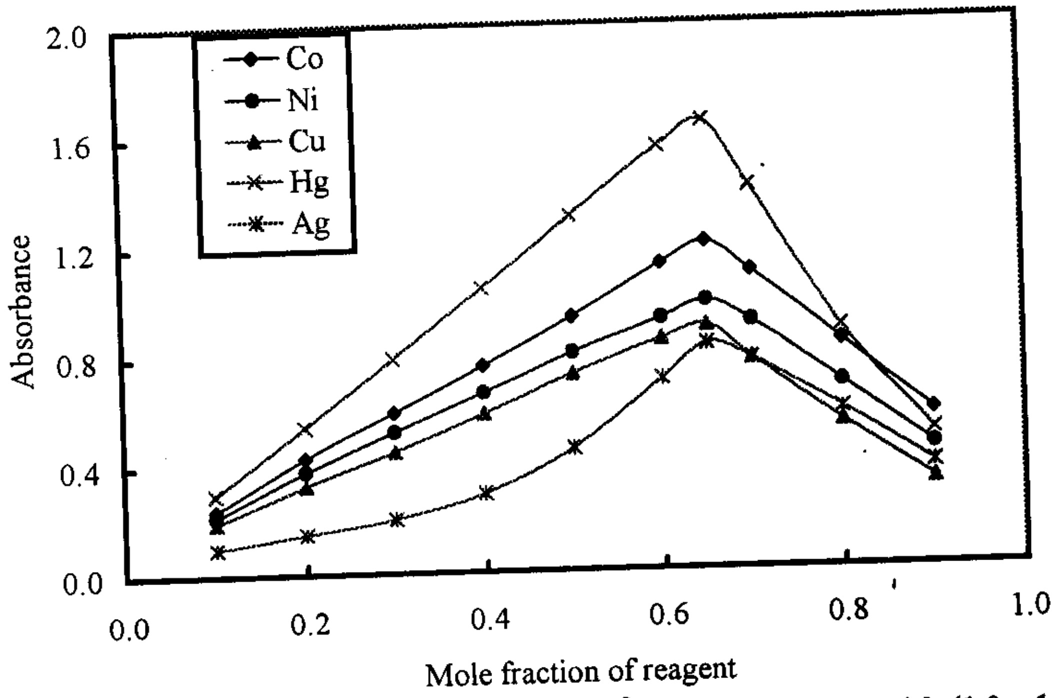


(a): Effect of reagent concentration on the absorbance of metal ions complexes using (2.0 x 10^{-3} M) of reagent R_6

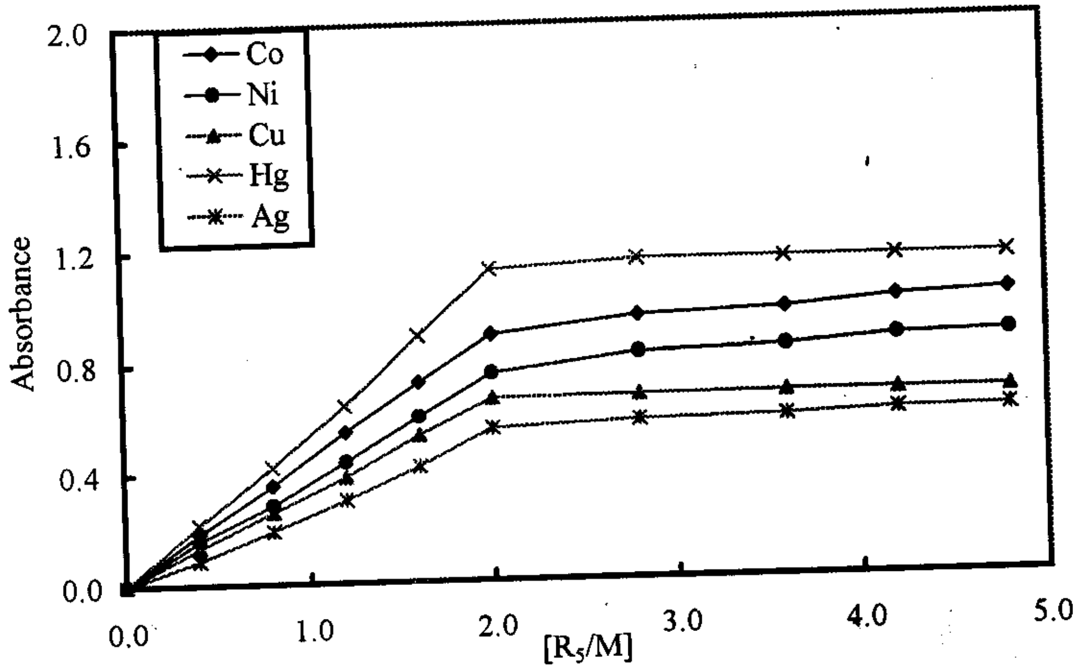


(b): Effect of reagent concentration on the absorbance of metal ions complexes in the presence of surfactant using (2.0 x 10^{-3} M) of reagent R_6

Fig. (41)

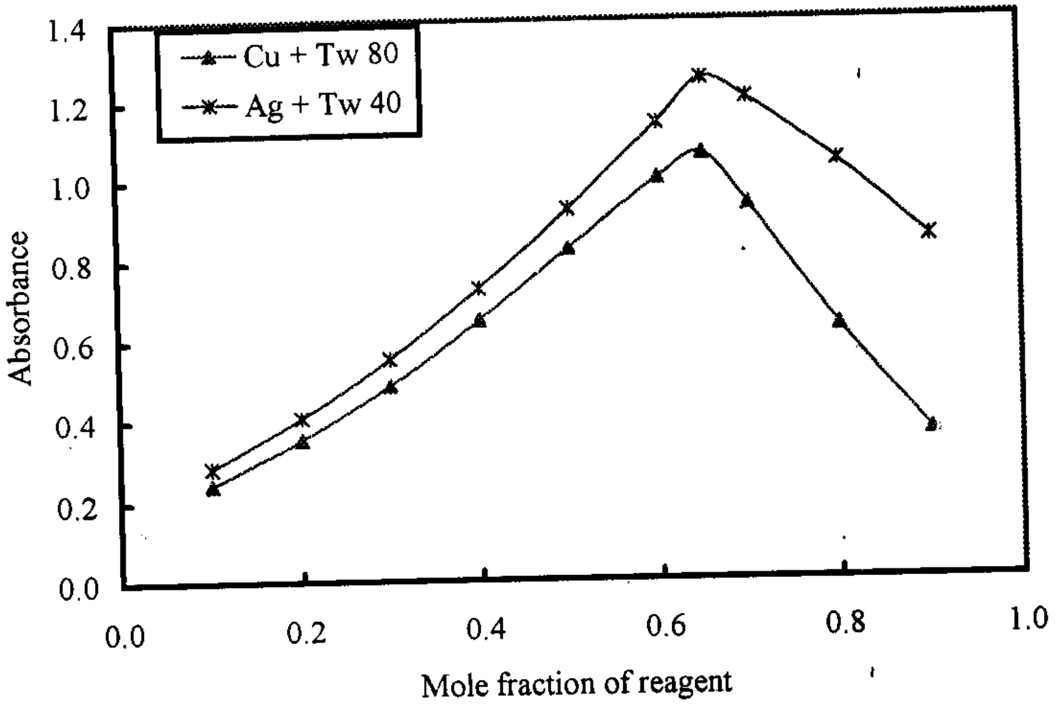


(a): Continuous variation using (1.0 x 10^{-3} M) of reagent R_5 with (1.0 x 10^{-3} M) of metal ions

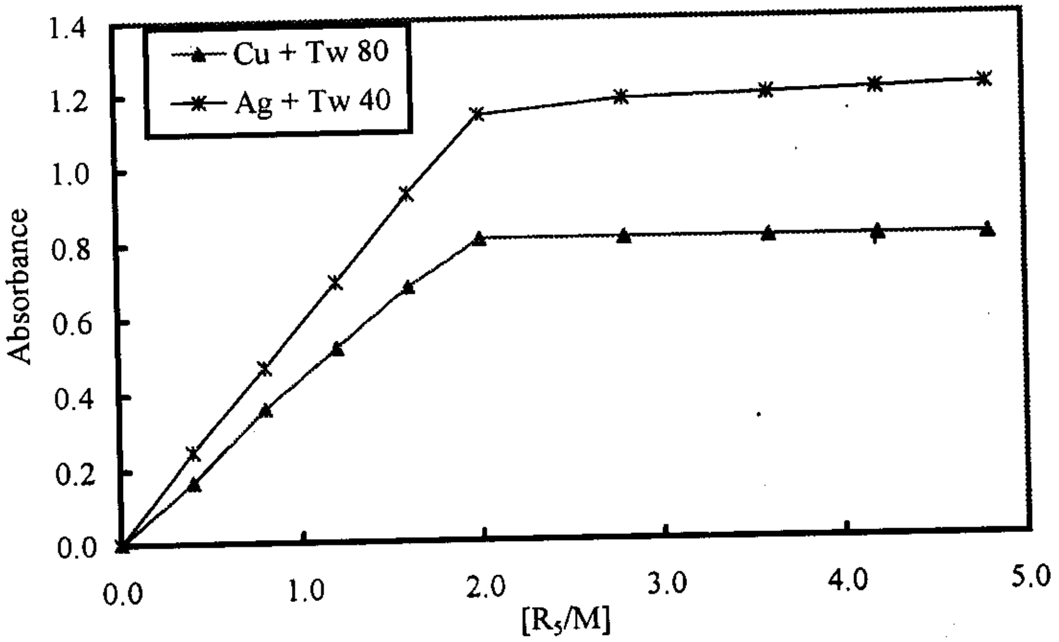


(b): Molar ratio using (1.0 x 10^{-3} M) of reagent R_5 with (0.5 ml of 1.0 x 10^{-3} M) of metal ions

Fig. (42)

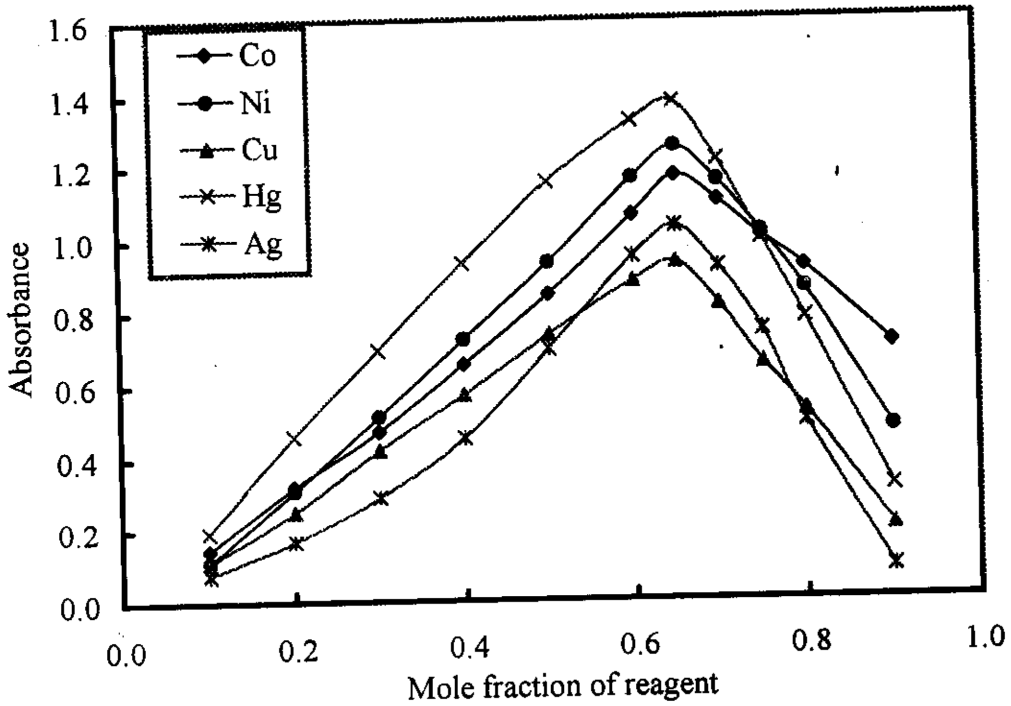


(a): Continuous variation using (1.0 x 10^{-3} M) of reagent R_5 with (1.0 x 10^{-3} M) of metal ions in the presence of surfactants

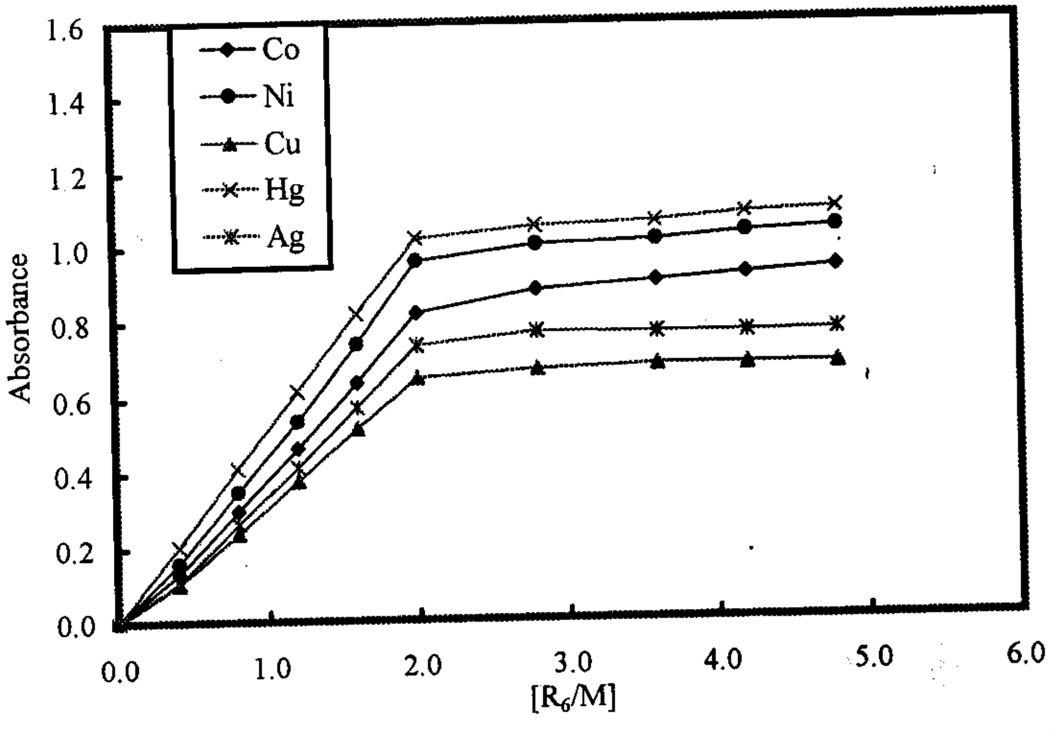


(b): Molar ratio using (1.0 x 10^{-3} M) of reagent R_5 with (0.5 ml of 1.0 x 10^{-3} M) of metal ions in the presence of surfactants

Fig, (43)

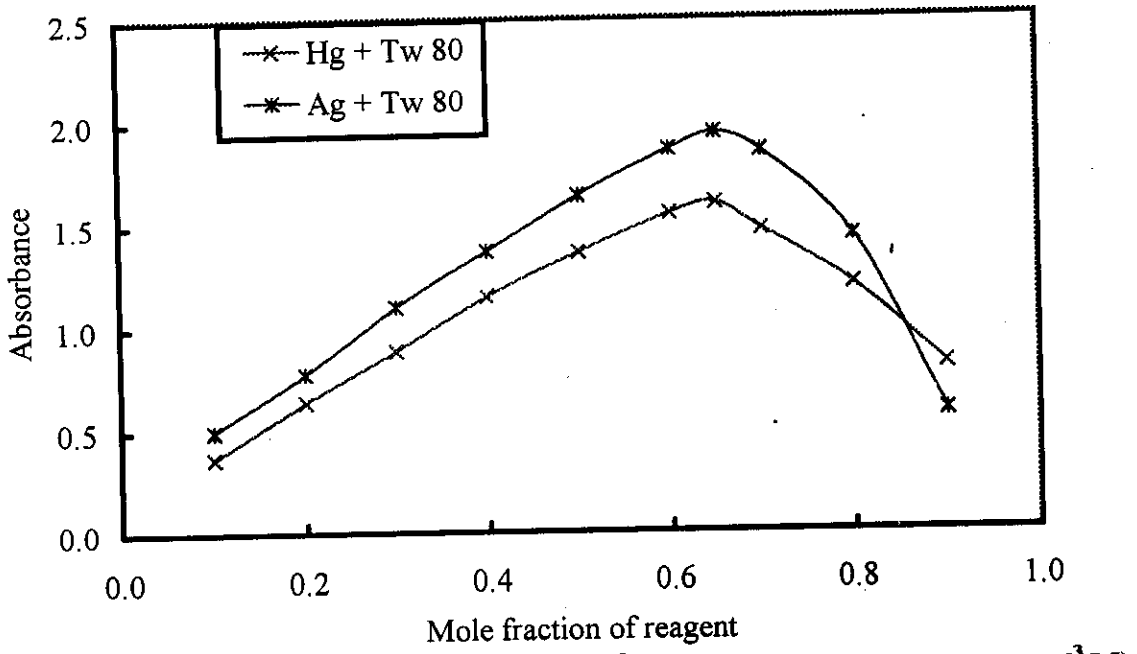


(a): Continuous variation using (1.0 x 10^{-3} M) of reagent R_6 with (1.0 x 10^{-3} M) of metal ions

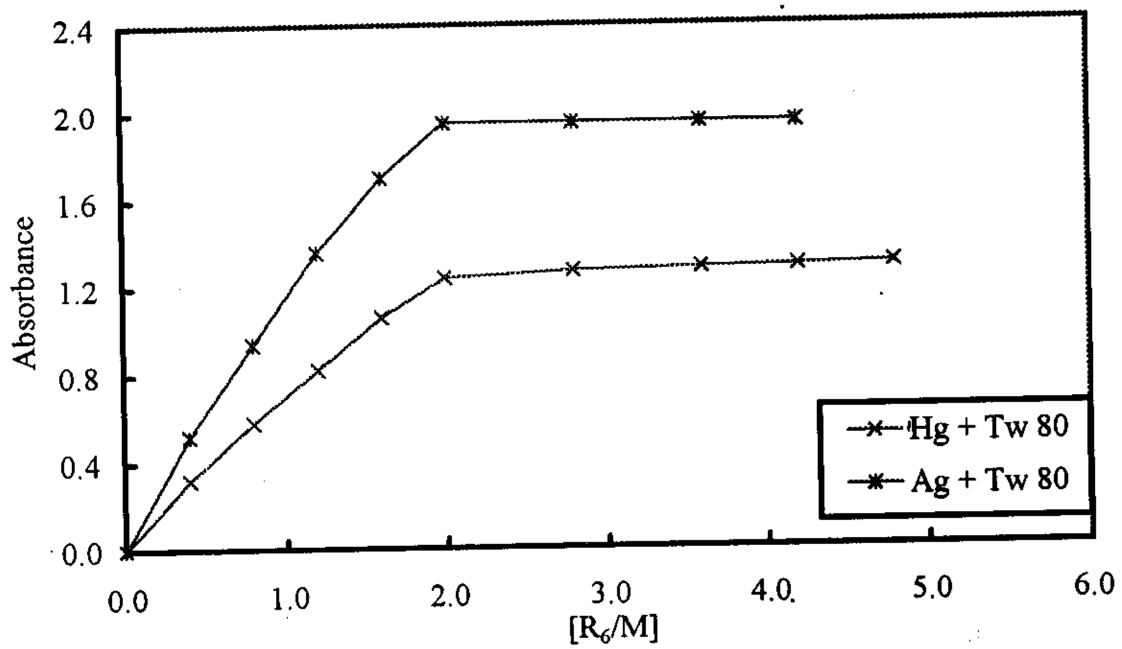


(b): Molar ratio using (1.0 x 10^{-3} M) of reagent R_6 with (0.5 ml of 1.0 x 10^{-3} M) of metal ions

Fig. (44)



(a): Continuous variation using (1.0 x 10^{-3} M) of reagent R_6 with (1.0 x 10^{-3} M) of metal ions in the presence of surfactant



(b): Molar ratio using (1.0 x 10^{-3} M) of reagent R_6 with (0.5 ml of 1.0 x 10^{-3} M) of metal ions in the presence of surfactant

Fig. (45)

Table (25): Optimum condition parameters of reagent R_5 with the ions Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^{+}.

Parameters	**R_5**						
	Co^{2+}	**Ni^{2+}**	**Cu^{2+}**		**Hg^{2+}**	**Ag^{+}**	
			Withou[S]	**With[S]**		**Withou[S]**	**With[S]**
Working pH	10.50	9.50	10.50	10.50	9.50	9.50	9.50
λ_{max} (nm)	535	530	536	543	559	523	532
Buffer volume	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
Reagent concentration (2.0×10^{-3} M)	1.5 ml	1.7 ml	1.0 ml	1.0 ml	1.0 ml	1.5 ml	1.0 ml
Surfactant type	----	----	----	Tween 80	----	----	Tween 40
Surfactant volume	----	----	----	1.5 ml	----	----	1.5 ml
Sequence of addition	R-B-M	R-B-M	R-B-M	R-B-S-M	R-B-M	R-B-M	R-B-S-M
Stoichiometric ratio (R:M)	2:1	2:1	2:1	2:1	2:1	2:1	2:1
Stability constant[a]	12.03	11.31	11.10	13.12	13.12	11.06	13.28
Stability constant[b]	12.21	11.42	11.48	13.41	13.60	11.33	13.09
Average of stability constant	12.12	11.37	11.29	13.27	13.36	11.20	13.19

R: reagent B: Buffer [S]: surfactant M: metal

[a]: Stability constant using molar ratio method

[b]: Stability constant using contanous variation method

Table (26): Optimum condition parameters of reagent R_6 with the ions Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^+.

Parameters	**R_6**						
	Co^{2+}	**Ni^{2+}**	**Cu^{2+}**	**Hg^{2+}**		**Ag^+**	
				Withou^S	**With^S**	**Withou^S**	**With^S**
Working pH	8.50	8.0	8.0	8.0	8.0	8.0	8.0
λ_{max}	543	550	555	558	558	546	556
Buffer volume	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
Reagent concentration (2.0×10^{-3} M)	1.0 ml	1.0 ml	1.2 ml	1.0 ml	1.5 ml	1.5 ml	1.0 ml
Surfactant type	----	----	----	----	Tween 80	----	Tween 80
Surfactant volume	----	----	----	----	1.5 ml	----	2.0 ml
Sequence of addition	R-B-M	R-B-M	R-B-M	R-B-M	R-B-S-M	R-B-M	R-B-S-M
Stoichiometric ratio (R:M)	2:1	2:1	2:1	2:1	2:1	2:1	2:1
Stability constant^a	10.65	11.04	11.22	12.90	14.43	11.30	14.41
Stability constant^b	10.52	11.17	11.66	13.07	14.14	11.46	14.87
Average of stability constant	10.59	11.11	11.44	13.49	14.29	11.39	14.64

R: reagent B: Buffer ^{S}: surfactant M: metal

^{a}: Stability constant using molar ratio method

^{b}: Stability constant using contanous variation method

3.5.3.9. Spectrophotometric determination of metal ions

a) Validity of Beer's law

The validity of ***Beer's*** law when reagents $\boldsymbol{R_5}$ and $\boldsymbol{R_6}$ are used for spectrophotometric determination of Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^{+} ions are shown graphically in Fig's (46-49). The limits of determinations of these metal ions are listed in Tables (27 and 28).The results show high sensitivity in microdetermination of the metal ions under investigation. This sensitivity is increased especially for Cu^{2+} and Ag^{+} complexes with reagent $\boldsymbol{R_5}$ when 1.5 ml of 0.5% (v/v) of Tween 80 and Tween 40 is added. Also the sensitivity of Hg^{2+} and Ag^{+} complexes with reagent $\boldsymbol{R_6}$ are incresed with the addition of 1.5 ml and 2.0 ml of Tween 80 respectively

For more accurate results ***Ringbom*** procedure is used by plotting log [M] in µg/ml against T%(Transmttance). The linear part of the S-shaped curve in Fig's. (46-49) gives the more accurate range of determination. The results are listed in Tables (27 and 28).

b) Evaluation of the accuracy and precision of the proposed method

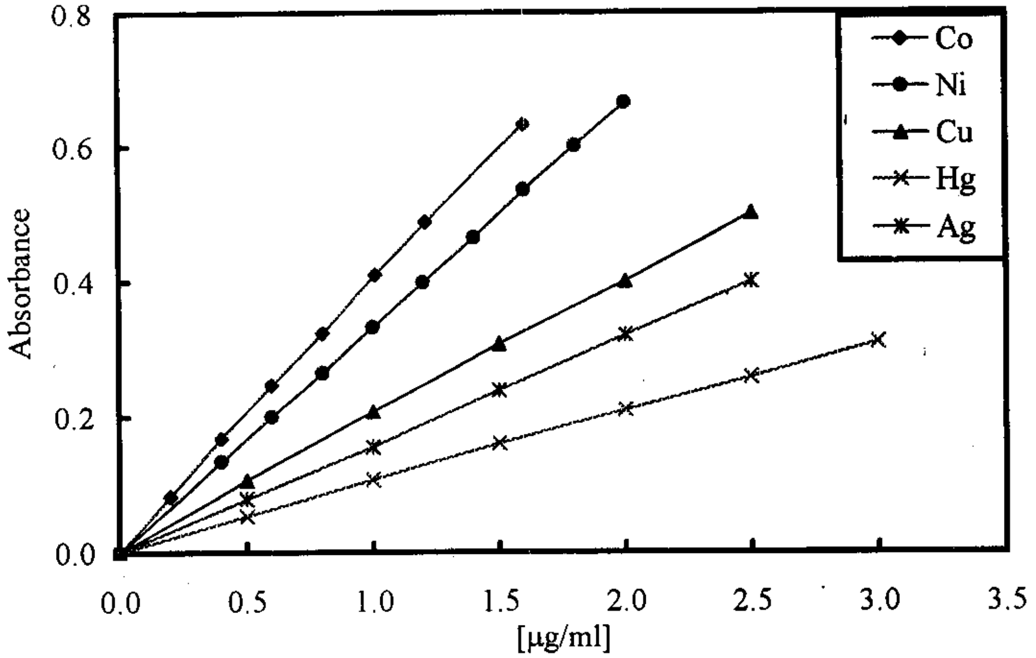
To estimate the accuracy and the precision of the proposed methods, solutions of metal ions containing two different concentration are analysed in six replicates by using reagents $\boldsymbol{R_5}$ and $\boldsymbol{R_6}$. The results obtained are collectted in Tables (29 and 30) show the ability of the proposed methods to determine low concentration levels of the metal ions under investigation with high accuracy.

c) Interferences

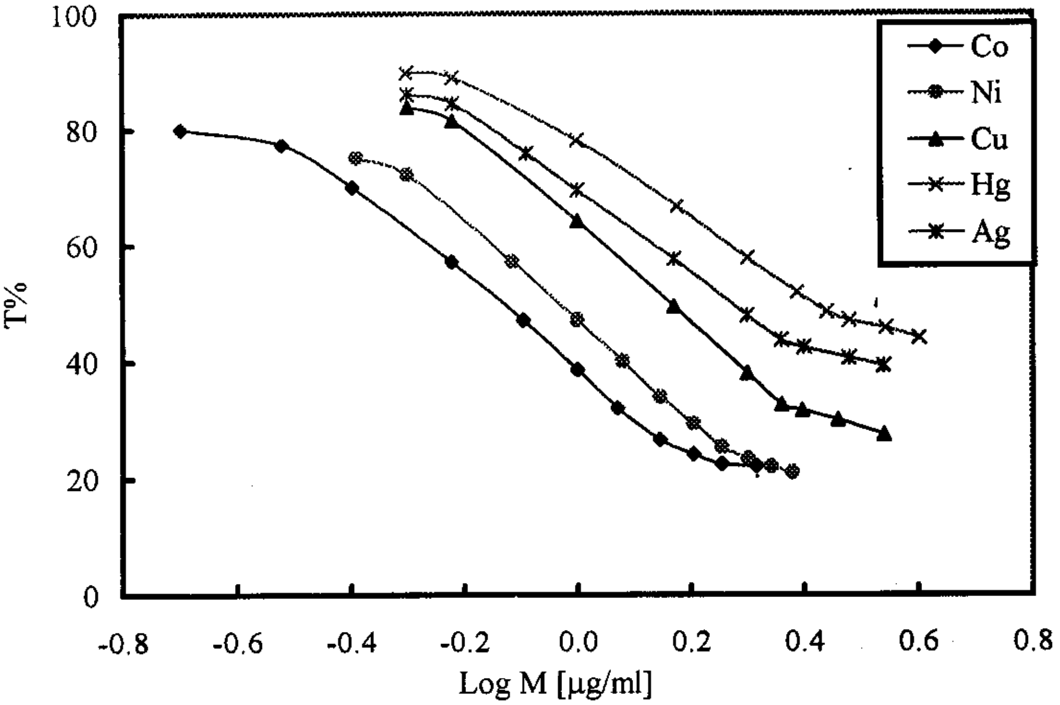
The effect of various cations and anions on the determination of the metal ions under considration is examined. The results show that, large amounts of Na^+, K^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Pb^{2+}, Bi^{3+}, Al^{3+}, La^{3+}, NH_4^+, HCO_3^- SO_4^{2-}, NO^-_3, VO^-_3, Mo^{6+}, W^{6+}, oxalate, tartarate, acetate, phosphate and borate. did not interfere whereas Zn^{2+}, Cd^{2+} and Fe^{3+} ions interfere, so it must be removed or masked.

3.5.3.10. Analytical application

The proposed method is successfully applied to the determination of the metal ions under investigation in their real samples by measuring different concentrations of the metal ions in µg/ml using the general procedure that is described for each metal ion. The standard method using atomic absorption spectrometry is also used as reference method. The results obtained as in Tables (31-32) show that the proposed method has high sensitivity in the microdetermination of metal ions. Also there is no significant difference between the proposed method and the offical method after calculating F value and t-test at 95% confedence limits and five degrees of freedom.

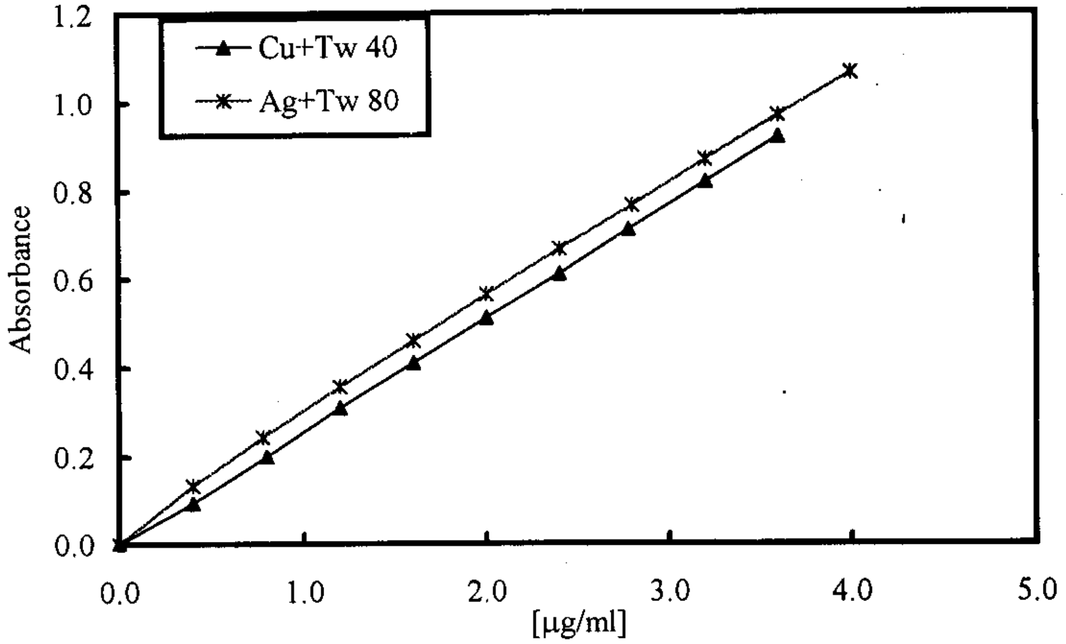


(a): Application of Beer's law for the studied metal ions complexes using the optimum volume (2.0×10^{-3} M) of reagent R_5

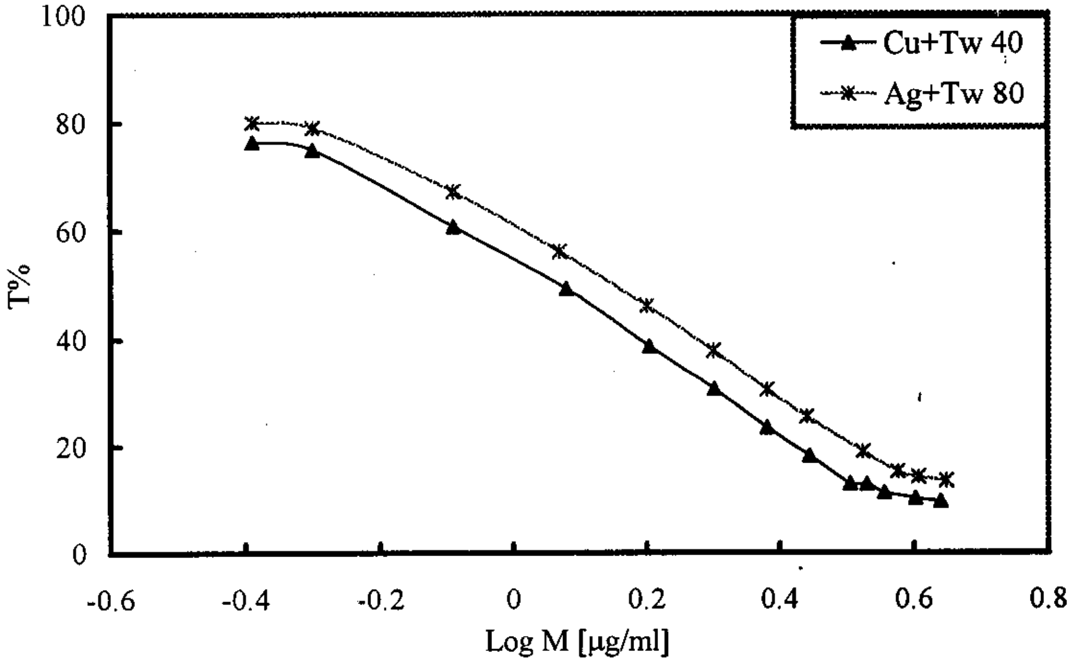


(b): Ringbom plots for the studied metal ions complexes using reagent R_5

Fig. (46)

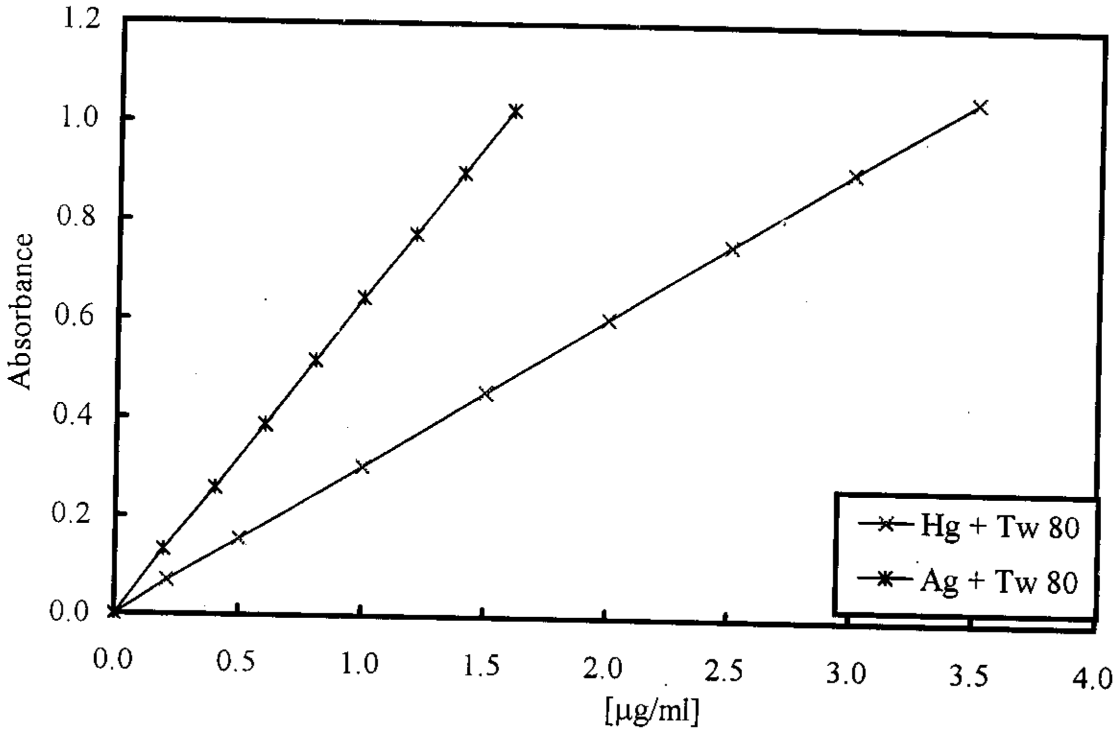


(a): Application of Beer's law for the studied metal ions complexes using the optimum volume (2.0 x 10^{-3} M) of reagent R_5 in the presence of surfactants

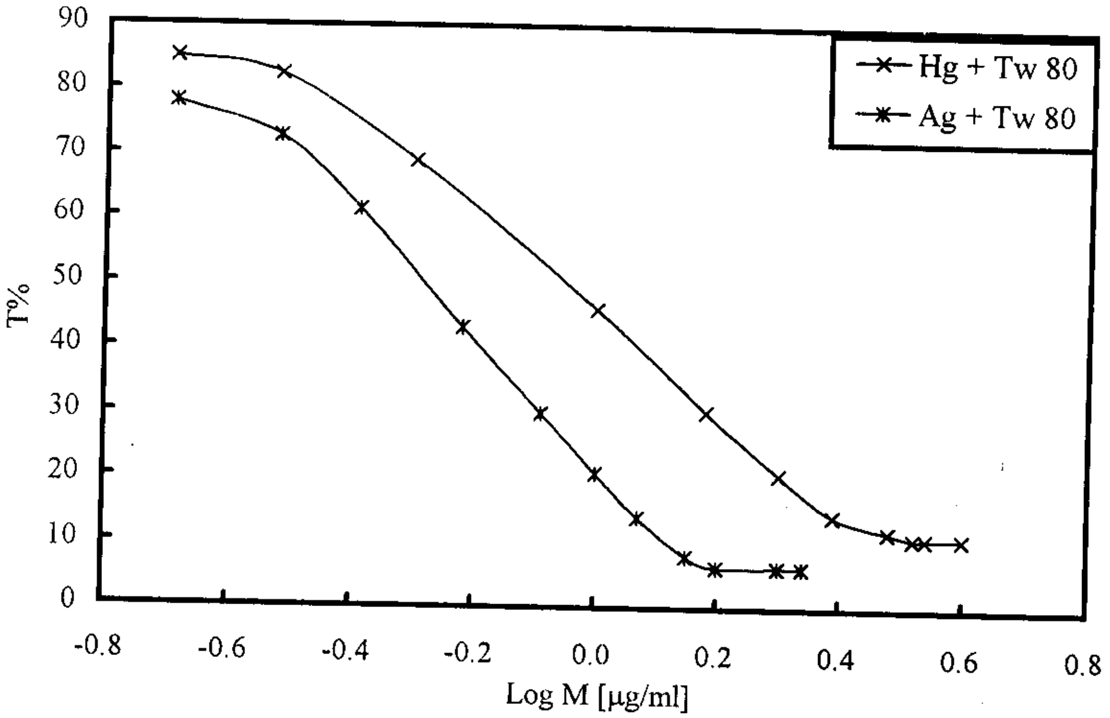


(b): Ringbom plots for the studied metal ions complexes using reagent R_5 in the presence surfactants

Fig. (47)



(a): Application of Beer's law for the studied metal ions complexes using the optimum volume (2.0 x 10^{-3} M) of reagent R_6 in the presence of surfactant



(b): Ringbom plots for the studied metal ions complexes using reagent R_6 in the presence of surfactant

Fig. (49)

Table (27): Analytical parameters of reagent R_5 complexes with Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^+ ions.

Parameters	**R_5**						
	Co^{2+}	**Ni^{2+}**	**Cu^{2+}**		**Hg^{2+}**	**Ag^+**	
			Without^S	**With^S**		**Without^S**	**With^S**
pH	10.50	9.50	10.50	10.50	9.50	9.50	9.50
λ_{max} (nm)	535	530	536	543	559	523	531
Beer's range (μg/ml)	0.2-1.6	0.4-2.0	0.5-2.5	0.4-3.6	0.5-3.0	0.5-2.5	0.4-4.0
Rangbom range (μg/ml)	0.3-1.4	0.5-1.8	0.6-2.3	0.5-3.4	0.6-2.8	0.6-2.3	0.5-3.8
Detection Limit (μg/ml)	0.038	0.049	0.076	0.055	0.120	0.082	0.049
Quantification limit (μg/ml)	0.127	0.166	0.254	0.183	0.401	0.274	0.165
SD*	0.0066	0.0058	0.0043	0.0052	0.0091	0.0052	0.0067
RSD %	1.43	0.757	0.408	0.909	0.638	0.253	0.496
Error %	0.245	0.204	0.163	2.04	0.367	0.204	0.245
Slope	0.359	0.332	0.199	0.257	0.103	0.160	0.236
Intercept	0.006	-0.001	0.005	-0.003	0.003	-0.001	0.009
Correlation Coefficient	0.9999	0.9999	0.9997	0.9999	0.9998	0.9998	0.9999
Molar absorptivity x 10^4 ($l.\ mol^{-1}\ cm^{-1}$)	2.12	1.95	1.26	1.63	2.07	1.72	2.55
Sandell Sensitivity (ng cm^{-2})	2.78	3.01	5.03	3.89	9.71	6.25	4.24

*: Average of six determinations

S: surfactant

Table (28): Analytical parameters of reagent R_6 complexes with Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Ag^+ ions.

Parameters	**R_6**						
	Co^{2+}	**Ni^{2+}**	**Cu^{2+}**	**Hg^{2+}**		**Ag^+**	
				Without^S	**With^S**	**Without^S**	**With^S**
pH	8.50	8.0	8.0	8.0	8.0	8.0	8.0
λ_{max} (nm)	543	550	555	558	558	546	556
Beer's range (μg/ml)	0.2-5.2	0.2-4.8	0.2-5.5	1.0-16	0.2-3.5	0.4-3.6	0.2-1.6
Rangbom range (μg/ml)	0.3-5.0	0.3-4.6	0.4-5.3	1.2-15.6	0.3-3.3	0.5-3.4	0.3-1.4
Detection Limit (μg/ml)	0.040	0.037	0.058	0.123	0.047	0.056	0.029
Quantification limit (μg/ml)	0.134	0.122	0.193	0.409	0.157	0.187	0.099
SD*	0.0051	0.0064	0.0082	0.0045	0.0049	0.0078	0.0084
RSD %	0.506	0.609	1.167	0.113	0.324	0.394	0.808
Error %	0.204	0.245	0.326	0.204	0.163	0.285	0.163
Slope	0.297	0.378	0.325	0.113	0.302	0.278	0.651
Intercept	-0.01	0.01	0.02	-0.008	0.01	-0.01	0.008
Correlation Coefficient	0.9998	0.9999	0.9999	0.9999	0.9998	0.9998	0.9999
Molar absorptivity x 10^4 ($l. mol^{-1} cm^{-1}$)	1.75	2.22	2.07	2.26	6.06	2.99	7.02
Sandell Sensitivity (ng cm^{-2})	3.37	2.65	3.08	8.85	3.31	3.60	1.54

*: Average of six determinations

S: surfactant