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A Highly Sensitive Indirect Spectrophotometric Method for the Determination of Some Phenothiazine Drugs

M.A. KARIMI*† and M.R. HORMOZI NEZHAD‡
Department of Chemistry, Payame Noor University of Ardakan, Ardakan, Iran
Fax: (98)(351)8253221 E-mail : m_karimi@pnu.ac.ir; ma_karimi43@yahoo.com

A new, simple, rapid, sensitive and accurate indirect spectrophotometric method for the microdetermination of some phenothiazine drugs in pure form and pharmaceutical formulations is developed. The proposed method is based on the reaction of chlorpromazine hydrochloride (CPH), promethazine hydrochloride (PH), trifluoperazine hydrochloride (TFPH), trimipramine maleate (TPM) and thioridazine hydrochloride (TRDH) with copper(II) and a subsequent reaction with neocuproine (NC). In the presence of NC, Cu(II) is reduced easily by phenothiazine derivatives to Cu(I)-NC complex, which shows an absorption maximum at 450 nm. The linearity ranges are found to be 0.3–150, 0.6–80, 0.5–190, 10–80 and 10–130 μg mL^{-1} for CPH, PH, TFPH, TPM and TRDH, respectively. This method was applied to the determination of these drugs in pharmaceutical formulations with recoveries in the range of 98.45–101.45%. The results obtained for the assay of pharmaceutical preparations compared well with those obtained by the official method and demonstrated good accuracy and precision.

Key Words: Phenothazine drugs, Spectrophotometric determination, Copper(II), Neocuproine.

INTRODUCTION

Phenothiazine derivatives which were introduced in the 1950's as antipsychotic drugs are still widely used in the treatment of moderate to severe mental illnesses including schizophrenia. Phenothiazines are also known for their antiemetic effects, the potency of the effects of anaesthetics, analgesics and sedatives and also as antihistamines[1]. More than 100 compounds derived from the fundamental phenothiazine skeleton have been synthesized and pharmacologically tested in the past five decades[2]. The vital importance of these drugs prompted the development of many analytical methods for their determination[3,4]. There are various analytical procedures for the assay of phenothiazines in body fluids as well as pharmaceutically and these methods have been reviewed[5–7]. Many spectrophotometric methods have been already proposed, but some of them either lack sensitivity and specificity[8–12], requiring long heating times[13–16] or involving non-aqueous media[17–20]. Some other spectrophotometric methods have

†Department of Chemistry, Shahid Bahonar University of Kerman, Kerman, Iran.
‡Department of Chemistry, Persian Gulf University, Bushehr-75168, Iran.

been used for the determination of phenothiazine drugs that have very narrow limits of detection[21-23].

The formation of the charge transfer complex between Cu(I) and neocuproine (NC) (2,9-dimethyl-1,10-phenanthroline) is the basis of the existing spectrophotometric method for the determination of trace amounts of reducing agents[24]. Reducing agents can be determined by reduction of Cu(II), followed by treating the Cu(I) with chromogenic reagent of NC. Recently, a rapid and sensitive spectrophotometric method for the determination of isoniazid based on its reducing ability has been reported[25]. Isoniazid can reduces the Cu(II)-neocuproine complex to the coloured Cu(I)-neocuproine complex.

This paper proposes a simple, rapid and sensitive indirect spectrophotometric method for the microdetermination of five phenothiazine drugs, *viz.*, chlorpromazine hydrochloride (CPH), promethazine hydrochloride (PH), trifluoperazine hydrochloride (TFPH), trimipramine maleate (TPM) and thioridazine hydrochloride (TRDH). These drugs can also reduce the Cu(II)-neocuproine complex to the coloured Cu(I)-neocuproine complex.

EXPERIMENTAL

A GBC UV-Vis Cintra 6 spectrophotometer model, attached to a pentium-IV computer, with 1 cm quartz cells was used for recording the absorbance spectra. Measurements of pH were made with a Metrohm 691 pH-meter using a combined electrode. All experiments were performed at 60°C. All reagents were analytical reagent grade. Triply distilled water was used throughout the study. Stock solutions (1000 μg mL^{-1}) of chlorpromazine hydrochloride, promethazine hydrochloride, trifluoperazine hydrochloride, trimipramine maleate and thioridazine hydrochloride (all from INC Biochemicals, USA) were prepared by dissolving 100 mg each of phenothiazine salts in distilled water and diluting to the mark in a 100 mL calibrated flask. These solutions were spectrophotometrically stable for at least 72 h. Working standards were prepared by appropriately diluting the above solutions with water. A stock solution of 5×10^{-3} M of neocuproine was prepared by dissolving 0.104 g of neocuproine (Merck) in 16 mL ethanol and diluting with distilled water in a 100 mL volumetric flask. A solution of 100 μg mL^{-1} of Cu(II) was prepared by dissolving 0.038 g of $Cu(NO_3)_2 \cdot 3H_2O$ (Merck) in water and diluting with water to the mark in a 100 mL volumetric flask. A buffer of pH 5 was prepared by using sodium acetate and hydrochloric acid at appropriate concentration.

Recommended procedure: A volume of 3 mL buffer solution (pH 5), 1.8 mL of stock neocuproine solution and 0.8 mL of 100 μg mL^{-1} Cu(II) solution were added to a 10 mL flask and made up to mark with water. For each measurement, 2 mL of the above solution was transferred to a spectrophotometer cell; then appropriate volumes of CPH, PH, TFPH, TPM and TRDH in the ranges of 0.3–150, 0.6–80, 0.5–190, 10–80 and 10–130 μg mL^{-1}, respectively, were injected to the cell by micro-syringe and absorbance was recorded at 450 nm after 3 min.

Procedure for tablets: Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder equivalent to 50 mg of phenothiazine salt was transferred into a 100 mL calibrated flask and diluted to volume with water. Using a mechanical stirrer, the powder was completely disintegrated and the solution was filtered. A suitable aliquot of this solution in the individual phenothiazine working range was treated as described in the recommended procedure.

Procedure for injections: An accurately measured volume was appropriately diluted to get 500 μg mL^{-1} of phenothiazine salt solution. A suitable aliquot of the solution was taken and the recommended procedure was followed for the analysis of drug content.

RESULTS AND DISCUSSION

The spectrophotometric method for the determination of phenothiazine derivatives is based on the oxidation reaction of the drugs with Cu(II) and subsequent reaction with neocuproine to form coloured Cu(I)-neocuproine product. The Cu(II)-neocuproine system allows the spectrophotometric determination of a reducing agent, A_{red}, provided that the redox reaction:

$$n\mathrm{Cu}^{2+} + 2n\mathrm{NC} + \mathrm{A}_{red} \longrightarrow n[\mathrm{Cu(NC)_2}]^{+} + \mathrm{A}_{ox}$$

is complete with the formation of an equivalent amount of $[Cu(NC)_2]^+$ with respect to the n-electron reductant, A_{red}. Cu(II) is a strongly oxidizing agent only when its reduction product, Cu(I), can form a colour complex by NC at 450 nm and make visible spectrophotometric signals for indirect determination of concentrations of phenothiazine drugs. The reduction of Cu(II) to Cu(I) in the presence of NC and subsequent complex formation between Cu(I) and NC takes a few minutes to complete. The absorbance reaches a maximum after about 2 min and remains constant afterwards. Therefore, all the absorbance measurements were performed 3 min after initiation of the reaction. The factors affecting the colour development, reproducibility, sensitivity and adherence to Beer's law such as pH, temperature, concentrations of NC and Cu(II) were studied to establish the best reaction conditions.

Absorption spectra: The reagent blank does not absorb in visible range of spectrum, but when CPH, PH, TFPH, TPM and TRDH react with $[Cu(NC)_2]^{2+}$, an orange-coloured cationic product, $[Cu(NC)_2]^+$, is formed which has an absorbance maximum at 450 nm. The absorption spectra of the products and reagent blank are shown in Fig. 1.

Effect of pH: The effect of pH on the reduction of Cu(II) by CPH, PH, TFPH, TPM and TRDH and formation of Cu(I)-neocuproine complex was studied over the pH range of 2.0–6.0. Fig. 2 shows the effect of changing pH on the absorbance of the solution mixture. The absorbance increases with increasing pH up to 5, thereafter remains constant. Therefore, pH 5 (acetate buffer) was selected for further studies.

Effect of neocuproine concentration: Fig. 3 shows the influence of neocuproine concentration on the absorbance in the concentration range of 5×10^{-5} to 3×10^{-3} M. As it is seen, at high concentrations of neocuproine, the absorbance due to Cu(I)-neocuproine complex decreases. This might be due to the fact that

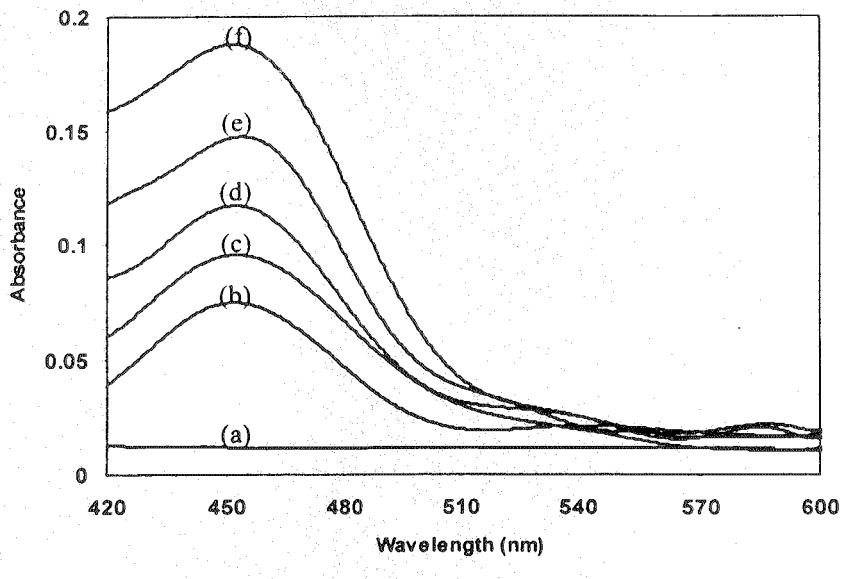

Fig. 1. Absorption spectra of: (a) Cu(II)-neocuproine reagent blank; Cu(I)-neocuproine complex in the presence of 10 μg mL^{-1}, (b) TPM, (c) TFPH, (d) TRDH, (e) CPH, (f) PH; 60°C temperature

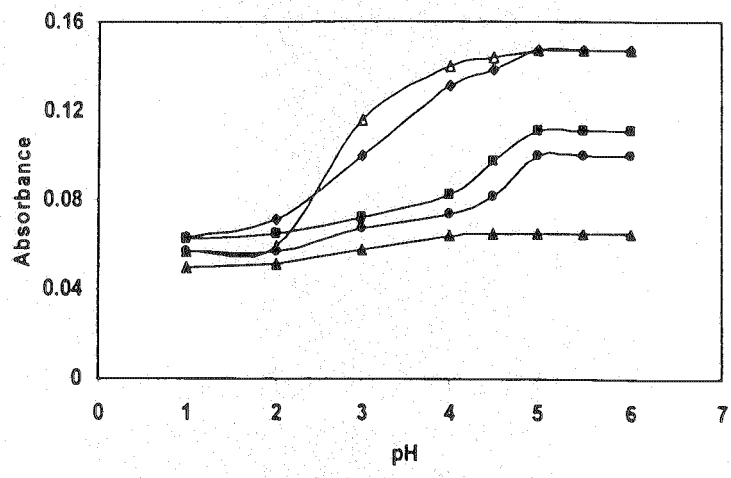

Fig. 2. Effect of pH on the absorbance of TPM (●),TFPH (▲),TRDH (Δ), CPH (♦), PH (■). Conditions: 10 μg mL^{-1} Cu(II); 1×10^{-3} M NC; 20 μg mL^{-1} phenothiazine drug; 60°C temperature

high concentrations of NC would result in a positive interference from Cu(II) that could have arisen from incomplete conversion of Cu(I) into Cu(I)-neocuproine complex *via* mixed ligand complex formation[25,26]. Thus, a concentration of 1×10^{-3} M NC was chosen as the optimum NC concentration.

Effect of Cu(II) concentration: The effect of Cu(II) concentration was examined over the range of 5–20 μg mL^{-1} of Cu(II) (Fig. 4.). The oxidizing power of Cu(II) in a solution containing NC is dependent on the ease of formation of $[Cu(NC)_2]^+$. An excess of Cu(II) can exhibit an affinity for NC[26]. Therefore, large excess of Cu(II) competes with Cu(I) for complex formation with NC. A 10 μg mL^{-1} Cu(II) concentration was selected as the optimum condition.

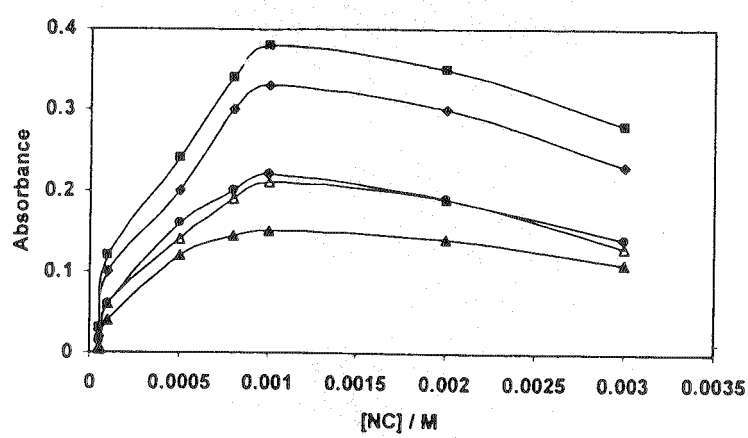

Fig. 3. Effect of neocuproine concentration on the absorbance of TPM (●),TFPH (▲),TRDH (Δ), CPH (♦), PH (■). Conditions: 10 μg mL^{-1} Cu(II); 20 μg mL^{-1} Phenothiazine drug; pH 5 (acetate buffer); 60°C temperature

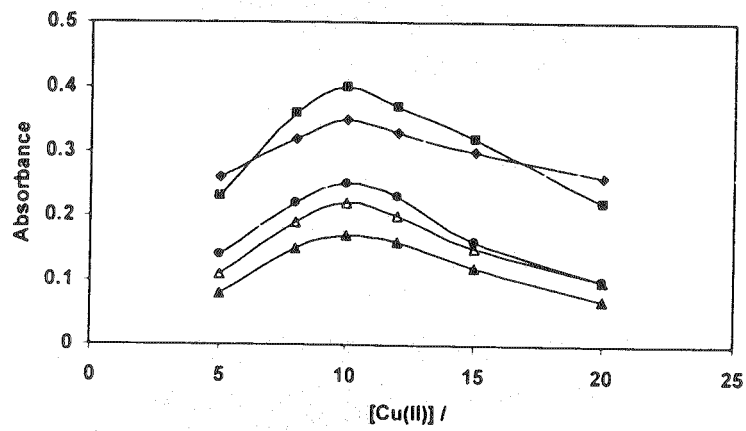

Fig. 4. Effect of Cu(II) concentration on the absorbance of TPM (●),TFPH (▲),TRDH (Δ), CPH (♦), PH (■). Conditions: 1×10^{-3} M NC; 20 μg mL^{-1} Phenothiazine drug; pH 5 (acetate buffer); 60°C temperature

Effect of temperature: The effect of temperature on the absorbance was studied in the range of 25–70°C. From the results, it is concluded that the absorbance increased with increasing temperature. Therefore, temperature of 60°C was chosen as the suitable temperature for further studies.

Reaction time and stability colour: The development of the coloured product was slow at room temperature. But the absorbance increased with increasing temperature. At a temperature of 60°C, a time of ≥ 2 min after the addition of drug is necessary for its reaction with Cu(II)-neocuproine before measuring the absorbance. After cooling to room temperature, the products were stable for 72 h.

Analytical parameters: A linear correlation was found between absorbance at λ_{max} for each drug and concentration in the range given in Table-1. Intercepts, slopes and corrrelation coefficients for the calibration data of the phenothiazine drugs are also presented in Table-1.

TABLE-1
ANALYTICAL PARAMETERS FOR THE SPECTROPHOTOMETRIC DETERMINATION OF PHENOTHIAZINE DRUGS

Parameter	CPH	PH	TFPH	TPM	TRDH
Colour	orange	orange	orange	orange	orange
λ_{max} (nm)	450	450	450	450	450
Stability (h)	72	72	72	72	72
Beer's law limits ($\mu g\ mL^{-1}$)	0.3–150	0.6–80	0.5–190	10–180	10–130
Detection limit† ($\mu g\ mL^{-1}$)	0.21	0.38	0.34	3.86	2.57
Molar absorptivity ($\times 10\ L\ mol^{-1}\ cm^{-1}$)	3.90	4.07	2.25	5.05	4.88
Sandell's sensitivity ($\mu g\ cm^{-2}$ per 0.001 A unit)	0.0091	0.0078	0.0213	0.0081	0.0083
Regression equation (A‡)					
Regression coefficient (r)	0.9972	0.9975	0.9990	0.9992	0.9989
Slope (b)	0.0110	0.0127	0.0047	0.0123	0.0120
Intercept (a)	0.1113	0.1090	0.5450	–0.0422	–0.0130

† Theoritical detection limit ($3S_b$ or three times of standard deviation of blank)[27].
‡ A= a + bC where "A" is the absorbance for concentration "C" in $\mu g\ mL^{-1}$.

Accuracy and precision: The accuracy of the method was established by analyzing the pure drug in three concentration levels and the precision by determining the relative standard deviation (RSD) of seven replicate analyses on the same solution containing three different concentration levels of each drug (Table-2).

TABLE-2
ACURACY AND PRECISION DATA

Phenothiazine derivative	Amount ($\mu g\ mL^{-1}$)		RSD (%) (n = 7)
	Taken	Found	
CHP	0.8	0.76	2.5
	20	21.10	1.8
	120	117.60	2.8
TFPH	1	0.96	2.0
	70	70.80	2.2
	160	158.60	2.6
3PH	3	2.96	1.9
	40	40.60	2.3
	80	79.40	2.9
TPM	12	11.90	2.5
	30	30.40	2.6
	70	68.90	2.1
TRDH	20	19.60	2.5
	75	76.40	1.7
	120	118.70	2.9

Interference studies: The interference by commonly associated excipients in pharmaceutical preparations such as talc, glucose, starch, lactose, dextrose, sodium alginate and magnesium stearate was investigated by preparing synthetic mixtures containing 20 μg mL^{-1} of each drug and 10-fold excess amounts of the excipients. The tolerance limit was defined as the concentration which gave an error of 3% or less in the determination of 20 μg mL^{-1} of drug. The results are presented in Table-3. From the results, it is concluded that the method is free from interference of excipient species. Only ascorbic acid appeared to interfere with drugs in this method. The interference of ascorbic acid was eliminated when the synthetic sample solution was measured after ≥ 1 h.

TABLE-3
RECOVERY OF 20 μg mL^{-1} PHENOTHIAZINE DRUGS FROM SOLUTIONS WITH A 10-FOLD CONCENTRATION OF VARIOUS ADDITIVES USED AS EXCIPIENTS

Additive	% Recovery of phenothiazine drug ± % RSD[a]				
	CPH	TPM	TRDH	PH	TFPH
Talc	102.0 ± 0.8	100.1 ± 0.9	100.4 ± 0.6	98.4 ± 1.2	99.5 ± 0.7
Glucose	101.7 ± 0.7	100.6 ± 1.3	98.0 ± 0.8	97.4 ± 1.4	100.8 ± 0.5
Starch	99.3 ± 1.1	98.6 ± 1.0	100.6 ± 0.5	98.2 ± 0.9	99.6 ± 1.5
Lactose	100.5 ± 1.4	102.2 ± 1.1	100.8 ± 0.7	99.5 ± 0.8	97.8 ± 1.4
Dextrose	97.0 ± 1.7	100.3 ± 0.9	100.8 ± 1.0	101.8 ± 1.5	100.4 ± 0.6
Sodium alginate	100.5 ± 0.7	101.8 ± 1.2	102.8 ± 1.6	100.8 ± 0.8	101.9 ± 1.3
Magnesium stearate	99.0 ± 1.2	101.4 ± 1.4	100.8 ± 1.1	101.2 ± 0.9	100.6 ± 1.0

[a]Average of four determinations

Application: The proposed method was successfully applied to the determination of CPH, PH, TFPH, TPM and TRDH in pharmaceutical preparations. The same samples were also analyzed by the British Pharmaceutical (BP) official method[28] and per cent recovery, standard deviation (SD), Student's t-test value and F-test value were calculated (Table-4). The results reveal that similar degrees of accuracy and precision are afforded by both methods.

Conclusion

This method is simple, rapid, fairly selective and much more sensitive than some of the reported methods (Table-5). The other advantages of the present method over the previous methods include rate of development and stability of the colour of product, wide range of determination without the need for extracting, low detection limit with high accuracy and precision. The high λ_{max} (in visible region) of the proposed method is a decisive advantage since the interference from associated excipients was not observed. Thus, the proposed method can be

used as an alternative for rapid and routine microdetermination of bulk samples and various pharmaceutical formulations.

TABLE-4
RESULTS OF DETERMINATION OF STUDIED DRUGS IN PHARMACEUTICAL FORMULATIONS

Drug and formulation[a]	Label claim (mg/tablet or mg/mL)	Found[b] (% recovery ± SD)		Student's t-value[c]	F-value[d]
		Proposed method	Official BP method		
CPH					
Tablet (1)	25	98.78 ± 0.97	99.23 ± 1.14	1.44	2.28
Tablet (1)	100	99.12 ± 0.68	100.76 ± 0.92	1.85	2.66
Injection (1)	25	100.65 ± 0.76	101.24 ± 0.82	2.17	3.74
TFPH					
Tablet (2)	1	98.50 ± 1.12	99.64 ± 0.89	1.48	1.75
Tablet (2)	5	99.02 ± 0.70	99.36 ± 0.58	2.44	3.14
Tablet (2)	10	98.84 ± 1.06	100.60 ± 0.81	1.95	2.55
Injection (2)	1	101.45 ± 0.70	100.34 ± 0.58	1.59	2.82
PH					
Tablet (1)	25	98.45 ± 0.48	99.56 ± 0.76	1.28	1.88
Injection (1)	25	99.02 ± 0.64	98.56 ± 0.64	1.94	1.48
TPM					
Tablet (1)	25	98.46 ± 0.52	99.42 ± 0.72	1.64	2.71
Injection (1)	100	100.68 ± 0.36	100.30 ± 0.43	1.80	1.65
TRDH					
Tablet (3)	10	98.74 ± 0.94	99.92 ± 0.82	2.20	4.24
Tablet (3)	25	101.06 ± 0.48	100.74 ± 0.54	2.08	2.76
Tablet (3)	100	99.04 ± 0.85	100.16 ± 0.42	1.96	1.48

[a]Marketed by: (1) Tehran Chimi, (2) Iran Daru Pakhsh and (3) Pars Minoo.
[b]Average of five determinations ± standard deviation.
[c]Tabulated Student's t-value at 95% confidence level is 2.78
[d]Tabulated F-value at 95% confidence level is 6.39.

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TABLE-5
COMPARISON OF SOME VISIBLE SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF PHENOTHIAZINE DRUGS

S.No.	Reagent	Coloured species	Drug analyzed	λ_{max} (nm)	Range of application (ppm)	ε ($L\ mol^{-1}\ cm^{-1}$)	Ref.
1.	Picrate	Charge transfer complex	PH	406	5–65	not reported	29
2.	N-Bromosuccinimide in strong sulfuric acid medium	Radical cation	PH, CPH, FPH	512–562	1–64	$(5.9–20.6) \times 10^4$	30
3.	Iron(III) and 1,10-phenanthroline	Complex formation	PH, CPH	510	1–6	not reported	31
4.	Haematoxyline with chloramine-T	Oxidative coupling	PH, CPH	555	4–23	$(4.4–7.5) \times 10^3$	32
5.	Brilliant blue orange II	Ion pair complex	PH, CPH, TFPH, FPH, PCPM	620	1–10	$(1.0–2.2) \times 10^4$	33
6.	1,2-Naphthoquinone-4-sulfonic acid	Radical cation	PH, CPH, FPH, TPH	490–510	2–30	$(3.2–6.0) \times 10^3$	34
7.	Iodic acid	Radical cation	PH, TPH, TFPH, FPH, PCPM	500–525	0.5–50	$(3.9–6.6) \times 10^3$	35
8.	Bromcresol green	Ion pair complex	CPH, TFPH, TPH, PCPM	420	2–16	$(1.7–2.6) \times 10^4$	36
9.	Iron and 3-methylbenzothiazoline-2-one hydrazone	Coupling product	CPH	720	2–20	1.9×10^4	37
10.	Chloramine-T in sulfuric acid medium	Radical cation	PH, CPH, TFPH, TPH, PCPM, TH	500–636	5–125	$(1.0–5.4) \times 10^3$	38
11.	Iron(III) and ferricyanide	Complex formation	PH, CPH, TFPH, FPH, PCPM, TPH, PDM	700–720	0.1–8	$(2.4–3.6) \times 10^4$	39
12.	Chloramine-T in hydrochloric acid medium and indigocarmine	radical cation	PH, CPH, TFPH, TH, PCPM, PCPMS	610	1–15	$(1.5–2.9) \times 10^4$	40
13.	Copper(II) and neocuproine	complex formation	PH, CPH, TFPH, TPM, TRDH	450	0.3–190	$(3.9–5.0) \times 10^4$	This work

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