Spectrophotometric determination of Chlorpromazine Hydrochloride in Pharmaceutical preparations

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Abstract

The present study includes analytical method for determination of the drug Chlorpromazine Hydrochloride (CPH) in some Pharmaceuticals using Molecular Absorption, in addition to investigating complexes obtained throughout. The analytical data obtained throughout this study could be summaries as follows: The optimal experimental condition for the chelate formation: (pH=1.8); concentration of Pd(II) (20 $\mu g.ml^{-1}$); reaction time (8 minutes); aqueous -to- organic phases (5:3); extraction time of complex (1.5 minute); Benzyl alcohol proved to be the best solvent for extraction of the complex CPH-Pd(II) without interference. λ_{max}= 459 nm metal -to-ligand (1:1); stability constant of complex CPH-Pd(II) (6.93×10^{8} M^{-1}).

Analytical figures of merits for determination of CPH using the developed procedure: Linear dynamic range (3-70)$\mu g.ml^{-1}$; Corrolation coffecient (r= 0.9993), Sandell Sensitivity (S=0.0452$\mu g.cm^{-2}$) ; D.L (0.13 $\mu g.ml^{-1}$); Erel.% (0.18%); RSD% (3.74%), Recovery % (100.2±0.42)%. Direct and standard addition methods were applied to both standards and specimens of pharmaceutical. Stability of complex was also investigated. For optimization of experimental condition, the response surface method (RSM) was applied and data obtained were found similar. This method has been applied to determination CPH in the well-known pharmaceutical Epichlor.

الخلاصة

يتضمن البحث استحداث طريقة تحليلية جديدة في تقدير المركب الدوائي هيدروكلوريد الكلوربرومازين CPH (المستعمل في علاج انفصام الشخصية) في مستحضر (Epichlor) بطريقة الامتصاص الطيفي الجزيئي ، تم تقدير الدواء بتكوين المعقد CPH-Pd(II) بعد تحديد الظروف العملية المثلى وهي الرقم الهيدروجيني(pH=1.8) وتركيز الأيون Pd(II) (20 ميكروغرام.مل$^{-1}$) ونسبة الطور المائي إلى العضوي (3:5) وزمن التفاعل 8 دقيقة لإكمال التفاعل قبل عملية الاستخلاص، أما افضل زمن للاستخلاص فهو دقيقه ونصف لاستخلاص المعقد الكلابي كله وافضل درجة حرارة هي درجة حرارة المختبر.

إن عملية الاستخلاص لمرة واحدة كانت كافية لاستخلاص المعقد. وجد أن Benzyl alcohol هو أفضل مذيب لاستخلاص معقد CPH-Pd(II) بدون تداخلات منشأ. وتم التقدير عند الطول الموجي (λ_{max}= 459 nm)، وتمت معرفة نسبة الاتحاد المولية بين الدواء والبلاديوم وهي (1:1)، وكذلك تم حساب ثابت الاستقرارية

المعقد (6.93×10^{8} مولاري$^{-1}$)، أما مديات التركيز في تعيين الدواء فكانت (3- 70 ميكروغرام.مل$^{-1}$) ومعامـل الارتباط (r = 0.9993) وحساسية ساندل (0.0452 مايكروغرام.سم$^{-2}$) والممتصية المولارية(7.86×10^{3} لتـر. مول$^{-1}$.سم$^{-1}$) وحد الكشف (0.13 ميكروغرام.مل$^{-1}$) والخطأ النسبي المئـوي (0.18%) والدقـة (3.74%) والاسترددية (100.2 ± 0.42)%. كما تم تعيين الدواء في المستحضر الصيدلاني(Epichlor) بالطريقة المباشرة وطريقة إضافات القياس .

Introduction

The discovery of the antipsychotic agent chlorproazine hydrochloride in the early 1950s and advent of even more powerful phenothiazinic psychopharmacological agent resent a landmark in the history of the medical and psychiatric sciences.

Chlorpromazine hydrochloride is the most important compound in the large group of phenothiazine derivatives. It is widely used as a therapeutic agent for treating various mental and personality disorders, in the prevention of vomit spasms and as an intravenous anti–hypertensive. Like other phenothiazines, it easily undergoes oxidation in acid medium under the action of many oxidizing agents leading to the formation of intensely colored oxidation products [1]. The oxidation process involves two subsequent and distinct one – electron steps. The first is reversible and result in the formation of a colored cation – radical and while the second, irreversible, giving rise to the colorless sulfoxid[2]. Due to their biomedical significance and the continuous introduction of these drugs ,the determination of phenothiazines, and in particular of chlorpromazine , has considerable interest and has induced many workers to explore new methods for their determination . The official methods for phenothiazines , listed in the British pharmacopoeia (BP) and US pharmacopoeia (USP) , consist in the non-aqueous potentiometric titrimrtry or spectrophotometry in the ultraviolet region [3,4] . A variety of ultraviolet methods have been reported and the available analytical techniques include: titrimertry with different electrodes or in aqueous phase [5–8] , spectrophotometry in the visible region after oxidation phenothiazine[9–16], spectrofluorimetry[17,18], chemiluminescence[19,20],high performance liquid chromatography[21–24] , differential pulse voltammetry [25], differential pulse polarography[26], differential pulse stripping voltammetry [27– 30] and electrophoresis [31 – 34] .Chromatographic techniques in combination with electrochemistry and mass spectrometry or fluorescence spectroscopy have also been reported [35] . This work can be applied successfully to pharmaceutical preparation containing chlorpromazine hydrochloride.

Experimental

Apparatus

- all spectral and absorbance measurements were carried out on a shimadzu UV-Visible 160 digital double - beam recording spectrometer .
- pH meter , Jenway 3020 .

Reagents

All chemicals used were of analytical reagent grade unless other wise state , chlorpromazine hydrochloride standard material and all Epichlor drugs was provided from the state company for drug industries and medical appliances samara – Iraq.

Chlorpromazine Hydrochloride Stock solution (1000 µg ml $^{-1}$)

A 0.1gm of CPH was dissolved in water (D.W) and diluted to 100 ml in a volumetric flask.

Palladium Stock solution (1000 µg ml $^{-1}$)

A 0.1666gm of $PdCl_2$ was disolved in 5ml of hydrochloride acid (2N) ,Diluted to100 ml in a volumetric flask with deionized water .

Analytical Procedures

(A) *Direct Calibration*

preparation of working calibration solutions in (3 – 70 µg CPH ml $^{-1}$): A volume in range of 15 – 350 µl of 1000 µg CPH ml $^{-1}$ transferred to (250 ml) separating funnels, then 1 ml of 100 µg Pd ml $^{-1}$ was added to each funnel and the pH of all solutions was adjusted to 1.8 using dil.HCl or NaOH solution. These solutions were set aside for 8 min at room temperature , and then diluted to 5 ml with DW. Each solution was extracted with 3 ml of benzyl alcohol after shaking for 1.5 min , then the absorbance of organic layer was measured at (λmax = 459 nm) against blank (organic solvent) . The calibration graph was constructed and unknown CPH concentration found by regression (Fig .1).

(B) *Standard additions*

An Appropriate equal volume of Drug samples solutions were add to 5 ml volumetric flask .An increase concentration of CPH standard solution plus 1ml of 100 µg Pd ml $^{-1}$ were added to each flask except one flask remain without standard addition . All solution was diluted to 5 ml with DW after pH adjusted. The content of each flask was transferred to separating funnel. Then extracted processes and measurement was applied as mentioned in (A) .the concentration of drug sample was obtained from the standard addition plot by regression (Fig 2).

Absorption spectra

I- drug stock solution

0.5ml of (1000 µg ml^{-1}) chlorpromazine hydrochloride standard solution , was transferred to 10 ml volumetric flask , and diluted to the mark with water ,4ml of this solution , was transferred to absorption cell , then the absorption spectrum of this solution was measured in the region between 200 to 600 nm using water as the reference.Fig (3) shows the two absorption maxima of drug was at 239 and 306 nm .

II – Palladium (II) stock solution

0.25 ml of (1000 µg ml $^{-1}$) Palladium (II) stock solution, was transferred to 5 ml volumetric flask , and diluted to the mark with water ,4ml of this solution , was transferred to absorption cell , then the absorption spectrum of this solution was measured in the region between 200 to 1100 nm using water as the reference. Fig (4) shows that a wavelength maximum of palladium (II) was at 235 nm .

III- orange-yellow complex of CPH with Palladium (II)

The absorption spectrum of extracted complex was measured in the region (200-800) using the extracting solvent as the reference. Fig (5) shows that a wavelength maximum was 459 nm.

Results and Discussion

Optimum Conditions

1-Effect of pH Values

The effect of pH on the formation of CPH-Pd(II) complex is shown in Fig.(6);from which it appears that the best pH is (1.8) for the formation of chelate complex .

2-Effect of Concentration of Palladium (II)

The concentration (20 μg ml $^{-1}$) of Palladium (II) was found enough for complete

formation of chelating complex , Fig (7) .

3-Effect of Reaction Time

Fig(8) refers that a reaction time of (8min) is enough for complete complex formation .

4–Organic Solvents used in the extraction

Since the method involves the measurement of complex in organic phase , it inecessary to use a solvent which will extract the chelate complex , but unreacted excess the Palladium (II) used. It was found the CPH is more soluble in warwe than in benzyl alcohol , but CPH-Pd(II) is more soluble in benzyl alcohol than water .

5- Effect of Extraction Time

Fig (9) reveals that the complex of CPH with Palladium (II) , needed (1.5 min) of shaking to reach a state of equilibrium .

6- Effect of Phase Ratio

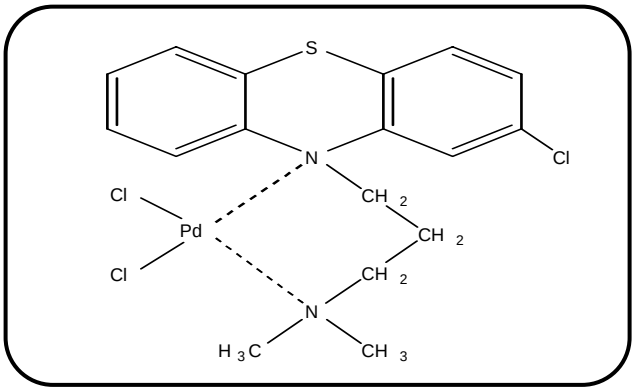
An aqueous – to – organic phase of 5:3 gives the highest extractability and better absorbance .

Extraction efficiency

Table (6) shows molecular absorbance values for the extracted chelating complex of CPH with Palladium (II) after the first and second extraction of the aqueouse phase . the extraction efficiency (E%) was found to be 97.29 and the distribution coefficient (D =59.83) was acheved .

Structure of the complex

Several techniques as FTIR, Molar ratio method have been used to elucidate the structure CPH-Pd(II) complex formed at optimal conditions and show Fig (10). The data revealed that a 1:1 complex.The data revealed that complex was formed with stability constant of 6.93×10^{8} M^{-1}, (λ_{max}= 459 nm) and from IR spectra and elemental analysis data, the following structure of the complex was suggested:



CPH-Pd(II)

The Response Surface Method (RSM) using Screening Design (SD) was also applied to estimate the effects of factors for the extraction of chelating

complex on statistical basis. Three main factors were selected such as the concentration of Palladium (II) ions (Cppm), the pH and volume of an aqueous phase (Vw). Table (1) shows the coding of these factors at two levels and Table (2) represents the 2^3-screeing design and factor levels for the estimation of the above mentioned factors. The factor effects were calculated as the difference between the responses of a factor at high and low level. These differences were then tested against the experimental error expressed by the standard deviation multiplied by the student's t-value. The factor effects were evaluated according to the relationships described elsewhere [36], and the results were shown in Table (3). Data have shown that the comparison of the experimental error with absoult differences reveal that the main factors pH and volume of aqueous phase show a significant effect (DpH and DVw are higher than 0.059), while the effect of Palladium (II) concentration can be neglected in the studied ranged between 20 and 60 μg ml^{-1} (i.e there is a minimal influence by the concentration of Palladium (II)). From the above study, the factors pH and Vw were found to be significantly influenced on the extraction of the chelating complex CPH-Pd(II) .A design at three levels,a Box- Behnken design was run at optimal Palladium (II) concentration in order to study the relationship between the response and the significant two factors. Table (4) shows the coding of the two factors at three levels, and Table (5) describes the factors at three levels according to Box-Behnken. The response surfaces were drawn graphically (Fig. 11 and 12). It can concluded that the curved dependences in the direction of both factors lead to a maximum absorbance at coded level of pH and Vw to the range close to the optimal values. Then, the surface startes to fall-off slightly in the case of increasing factor value from the optimal limit. However , the response surface was observed to be depressed extremely toward the least factor value , hence , inferring that it is necessary to maintain the pH at level higher than 1.1 and lower than 4.2 , and the same situation for volume of aqueous phase.

Calibration Graph

Fig (1) shows a calibration graph of CPH established by plotting the absorbance of complex vs. concentration and shows that beer's law is obeyed over the CPH concentration of (3 - 70 $\mu g ml^{-1}$) at wave length (459 nm) .

Statistical Calculations

All measurment can be characterized statistically . Table (7) shows the linear range of CPH-Pd(II) and detection limit , molar absorptivity (ε) , sandell sensitivity (s) and confidence limits for the concentration and the absorbanse .

Table (8) reveals that the test statistic t =96.33 is higher than critical value (2.16) in regression analysis (r =0.9993) . this means that the predications based on the estimated regression line Y = 0.01906X+0.01722 shoud be acceptable.Therefore , all concentration of CPH in the analyzed sample was determind from this relationship .

Table (9) shows the accuracy test in term of recovery . Recovery % was shown to be acceptable and found to be 100.2 ± 0.42 . Good precision as E_{rel} of the method was achieved and found to be 0.18 % .

Standard additions procedure was also applide (Fig .2) for the determination of CPH complex and all the analytical performances were tabulated in table (10). The two samples of direct calibration and standard addititions calculated was equal one , indicating the absence of interference

effects and use of direct calibration is to be preferred .

Analysis of CPH in pharmaceutical preparations with Palladium

Two procedures (direct calibration and standard additions) wer used to determine CPH in phenergan tablets at λ = 459 nm . The results were shows in table (11) and table (12) . Good agreement in concentration for both calibration was obtained compared with the stated concentration of 10 mg per unit .

Conclusions

This study has shown that the method described allows the rapid determination of Chlorpromazine Hydrochloride. The analytical scheme of the proposed system is simpler than that of other conventional procedures. Moreover, it offers a higher sensitivity compared with other analytical methods and better recovery.

The analytical results obtained for the determination of CPH in pharmaceuticals have shown good agreement with the given labeled quantity. The complex formed have stoichiometric ratio of 1: 1.

Table (1): Coding factors at two levels

Factor	+1	-1
PH	4.2	1.8
Vw	4	2
C_{ppm}	60	20

Table (2): 2^3- Screeing design and factor levels for estimation of the factors pH-values, the volume of aqueous phase and the concentration of palladium (II).

Coded Factor Level							
Run	**pH**	**Vw**	**C_{ppm}**	**pH C_{ppm}**	**pH. Vw**	**C_{ppm}.Vw**	**Response (Absorbance)**
1	-1	+1	+1	-1	-1	+1	0.410
2	-1	-1	+1	-1	+1	-1	0.64
3	-1	-1	-1	+1	+1	+1	0.641
4	-1	+1	-1	+1	-1	-1	0.407
5	+1	+1	+1	+1	+1	+1	0.11
6	+1	-1	+1	+1	-1	-1	0.550
7	+1	-1	-1	-1	-1	+1	0.520
8	+1	+1	-1	-1	+1	-1	0.500

Table (3): The comparison of the experimental error with the absolute Differences

Factor	Value	t(95% C.I, n=4) S.D
DpH	0.512	0.059
DVw	0.372	0.059
DC_{ppm}	0.022	0.059
$DpH.C_{ppm}$	0.017	0.059
DpH.Vw	0.232	0.059
DC_{ppm}.Vw	0.037	0.059

Table (4) : Coding the two factors at three levels

Factor	Level		
	+1	0	-1
pH	4.2	1.8	1.1
Vw	4	2	1

Table (5): Factor levels and Box-Behnken design for studing the CPH determination by spectrometric

Box-Bbehnken level			Response (Absorbance)
Run	pH	Vw	
1	+1	+1	0.600
2	+1	-1	0.650
3	-1	+1	0.600
4	+1	0	0.800
5	-1	0	0.610
6	0	+1	0.683
7	0	-1	0.805
8	-1	-1	0.805
9	0	0	0.915

Table (6) : absorbencies of complex after the first and second extraction

CPH ($\mu g.ml^{-1}$)	*Pd(II)* ($\mu g.ml^{-1}$)	*pH*	A_1 *(Ex. No.1)*	A_2 *(Ex. No.2)*	A_o *Blank*
45	20	1.8	0.902	0.047	0.006

Table (7) : analytical characteristics of result

λ_{max} (nm)	Linearity ($\mu g.ml^{-1}$)	D.L.*** ($\mu g.ml^{-1}$) (n=11)	D.L.T** ($\mu g.ml^{-1}$)	S ($\mu g.cm^{-2}$)	Conf. Limit. Conc.($\mu g.ml^{-1}$) 95% C.I	Conf. Limit. Abs. 95% C.I	ε ($L.mol^{-1}.cm^{-1}$)
459	3-70	0.13	0.218	0.0452	29.80±0.158	0.5854±0.00326	7.86×10^3

*** *Experimental*

** *Theoretical*

Table (8) : Regression equation , correlation coefficient (r) two tailed t-test and confidence limit for the slope for the intercept at 95% confidence level and (n – 2) degree of freedom for the calibration graph .

Regre. Eq. Y=BX+A	Corr. Coef. (r)	t- test statistic	Tabulated t- test two tailed (n-2) 95% C.I	Conf. Limit. for the slope $b + t_{sb}$	Conf. Limit for the intercept $a + t_{sa}$
Y=0.01906X+0.01722	0.9993	96.33	2.160	0.01906±0.0159	0.01722±0.0065

Table (9) : shows the relative standard deviation RSD% ,$E_{rel\%}$, recovery Rec%

Amount of CPH taken ($\mu g.ml^{-1}$)	Amount of CPH found ($\mu g.ml^{-1}$)	%Rec.	%Erel.	%RSD n = 5	Mean %Rec.+S.D	Mean %Erel.
5	4.9	98	-2	5.27	100.2+0.42	0.18
30	30.6	102	2	3.60	---	---
60	60.33	100.55	0.55	1.55	---	---

Table (10): shows regression equation , correlation coefficient (r) two tailed t-test and confidence limit for X – Value obtained (X_E) at 95% confidence limit and (n – 2) degree of freedom for the standard additions calibration graph , recovery Rec% , $E_{rel\%}$.

Regre. Eq. Y=BX+A	Corr. Coef. (r)	t- test statistic	Tabulated t- test two tailed n-2 95% C.I	Conf. Limit for x-value XE+tsXE	Rec. (%)	Erel. (%)
Y=0.0178X+0.174	0.9990	77.42	2.201	9.77±0.443	97.7	-2.3

Table (11): determination CPH in sample of pharmaceutical preparation by direct calibration and standard additions .

Name of pharmaceutical	Type of Preparation	Stated concentration (mg per unit)	Found (direct calb.) (mg per unit)	%Erel.	Found (st. add. calb.) (mg per unit)	%Erel.
Epichlor	Tablets	10	9.81	-1.9	9.77	-2.3

Table (12): shows the RSD% ,$E_{rel\%}$, recovery Rec% the calibration graph .

Amount of CPH taken ($\mu g.mL^{-1}$)	*Amount of CPH found ($\mu g.mL^{-1}$)*	*Rec. (%)*	*Erel. (%)*	*RSD (%) (n=5)*	*Mean Rec.%+S.D*	*Mean Erel. (%)*
5	5.18	103.60	3.60	2.80	102.14+0.39	2.14
30	31.10	103.33	3.33	1.87	---	---
60	59.70	99.50	-0.50	1.29	---	---

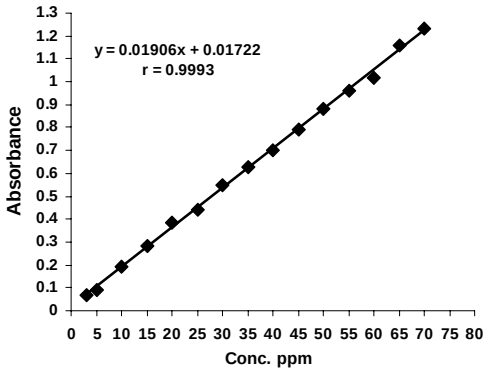


Fig (1) Calibration graph for the determination of CPH – Pd(II)

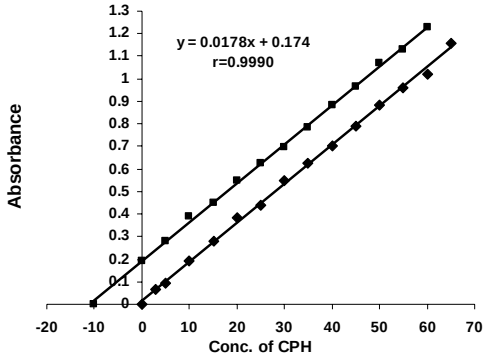


Fig (2) Determination of CPH in pharmaceuticals by using direct and standard additions procedures

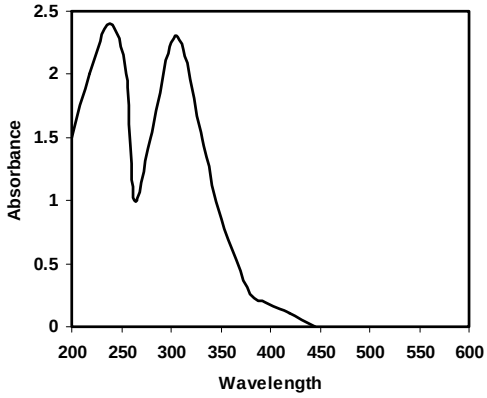


Fig (3): absorption spectrum of CPH

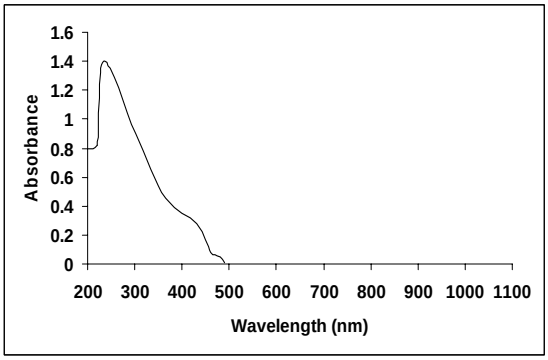


Fig (4): absorption spectrum of Pd (II)

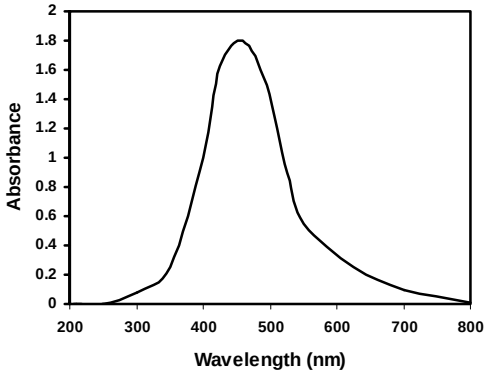


Fig (5): absorption spectrum of CPH-Pd(II)

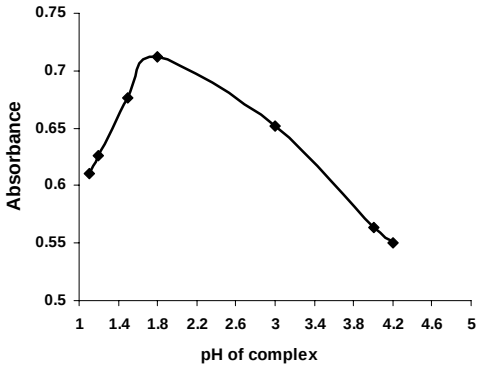


Fig. (6): Effect of pH

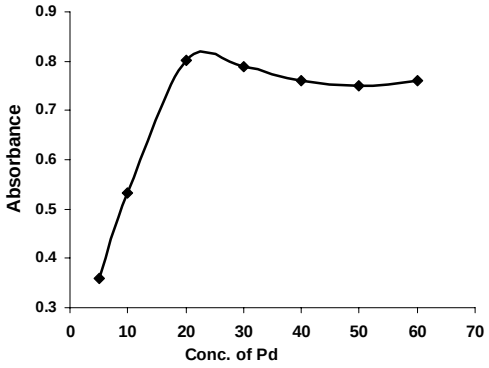


Fig (7) Effect of Con. of palladium on the determination of CPH

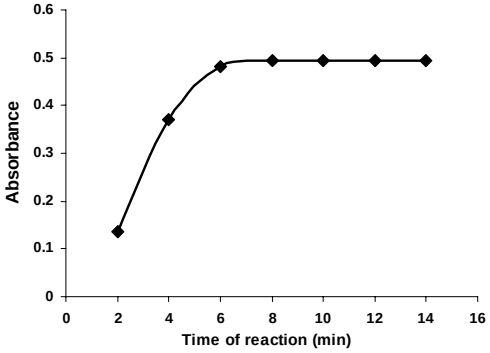


Fig.(8): Effect of reaction time

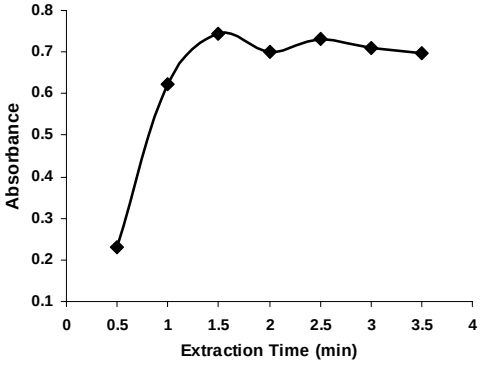


Fig (9) Effect of extraction time

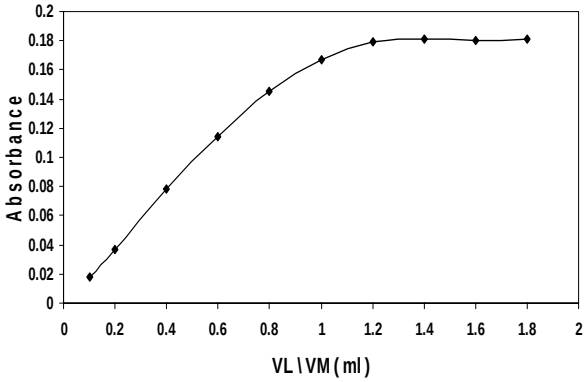
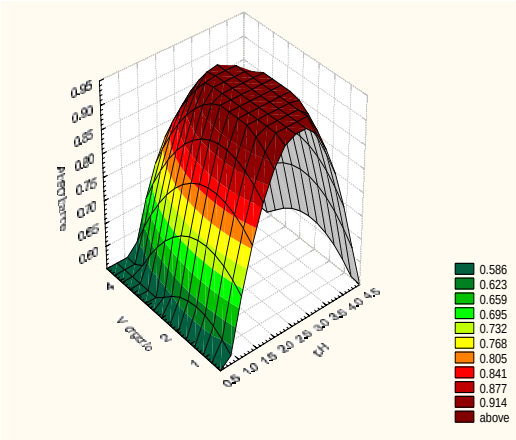
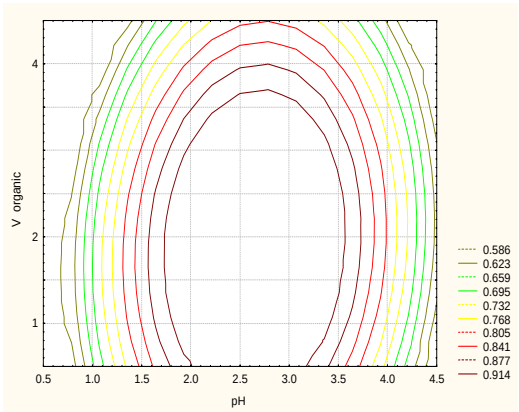


Fig (10) Molar ratio for CPH-Pd(II)



Fig(11) Screeing surface plot of absorbance versus the factors pH and volume of aqueous phase



Fig(12) Contour plot of absorbance versus the factors pH and volume of aqueous phase

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