Flow-injection spectrophotometric determination of chlorpromazine HCl based on releasing of sodium persulphate from hydrogel beads, study and applications

Issam.M.A.Shakir and Mohammed.J.Hamzah Al-kaffiji*
Chemistry Department -College of Science- Baghdad University
*Pharmacy College-Pharmaceutical chemistry Department- Kerbala University

التقدير بالحقن الجرياني الطيفي لدواء الكلوربرومازين هايدرو كلورايد بالاعتماد على تحرير بروسلفات الصوديوم من حبيبات بلورة الماء ,دراسة وتطبيق

عصام محمد علي –قسم الكيمياء – كلية العلوم – جامعة بغداد
*محمد جاسم حمزه- قسم الكيمياء الصيدلانية –كلية الصيدلة – جامعة كربلاء

المستخلص

تم تطوير أسلوب جديد للتحليل بالحقن الجرياني الطيفي لتقدير دواء الكلوربرومازين هيدروكلورايد في الصيغة النقية والمستحضرات الصيدلانية بالاعتماد على تحرر العامل المؤكسد صوديوم بيرسلفات من حبة بلورة الماء لغرض أكسدة الدواء وتكوين ناتج ذو لون وردي. كما تم استخدام منظومة مصنعة محليا للتحليل بالحقن الجرياني, حبة بلورة ماء واحدة وثنائي الوصلة باعث ذي اللون الأخضر كمصدر للتشعيع. الظروف المثلى التي تم التوصل إليها تتضمن 1مل.دقيقة$^{-1}$ كسرعة جريان, 100مايكوليتر حجم أنموذج محقن, 15ثانية زمن حقن الأنموذج, 0.5مول.لتر$^{-1}$ صوديوم بيرسلفات و 3ساعات زمن غمر حبة بلورة الماء في محلول بيرسلفات الصوديوم. تم الحصول على علاقة لتغير الاستجابة الإلية مع تركيز الدواء ضمن حدود تتراوح بين 0.17-0.8مول.لتر$^{-1}$ إما حد الكشف فوجد أنة مكافئ إلى 0.108 x 10^{-8} مللي مول.لتر$^{-1}$ من التخفيف التدريجي لاقل تركيز كما بلغ معامل الارتباط(r) 0.9945 . طبقت الطريقة بنجاح لتقدير الدواء في المستحضرات الصيدلانية وتبين من اجراء اختبار t المزدوج أنه لايوجد فرق جوهري بين الطريقة الجديدة والطرق المطبقة وعلى هذا الأساس يمكن استخدام الطريقة الجديدة كطريقة تحليلية بديلة لتقدير الدواء.

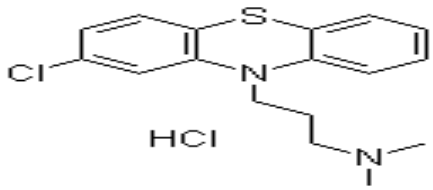
Abstract

A new mode for the FIA method was developed for the estimation of Chlorpromazine HCl(CPH) in pure and pharmaceutical formulation based on the releasing of the oxidizing agent sodium persulphate from the body the hydrogel beads leads to oxidation of drug to pink color product. Homemade FI photometer, single hydrogel bead and green light emitting diod were used for this application. The optimum conditions were 1ml.min^{-1} flow rate, 100µL injected sample volume, 15sec as purge time of injected sample volume, 0.5mol.L^{-1} of sodium persulphate that gel bead soaked in and 3hours of soaking time for hydrogel bead inside sodium persulphate solution. The linear range for the variation of instrument response with CPH concentrations was 0.17-0.80mmol.L^{-1}, While the limit of detection (L.O.D) was 0.108x10^{-8}M from the stepwise dilution of the minimum concentration in the linear

range. The correlation coefficient(r) was 0.9945. The method was applied successfully for the determination of CPH in pharmaceutical preparation, and paired t-test indicate that there was no significant differences between the new method and the reported method , so on that base the new method can be used as an alternative analytical method.

Introduction

Chlorpromazine HCl is a dimethylamine derivative of phenothazine, of a chemical formula of 2-chloro-10-[-3-(dimethylamino)propyl]phenothiazine monohydrochloride. It has the following chemical structure (fig(1)(1-3).



Fig(1): Chemical structure of chlorpromazine HCl.

Chlorpromazine HCl occurs as white or slightly creamy white, odorless crystalline powder which darkness on prolonged exposure to light (4-6). The principal pharmacological actions of chlorpromazine HCl are psychotropic. It also has a sedative and antiemetic activity. Chlorpromazine HCl has it's action at all levels on the central nervous system (CNS),primarily at sub-cortical levels as well as on multi-organs system(7). Numerous methods are available for the determination of chlorpromazine HCl,these include spectrophotmetry(8-21),Titrimetry(22-27),Potentiometry(28), electrochemical sensors(29), conductimetry(30), voltametry(31) and high-performance liquid chromatography(32).

Three-dimensional super absorption polymers were used as a absorbents for heavy metal ions from water and other aqueous solution susficialy. Polymer like polyacrylic acid, polyacryliamide and its derivatives which have functional groups (such as carboxylic, hydroxyl and amide) can be used as absorbents for metal ions removal via the interaction between the metal ions and these groups (33). Recently chlorpromazine HCl was determination spectrophotometrically(34,35) using various chromogenic reagents.

In this paper polyacrylic acid hydrogel bead was used as a host for the oxidant (sodium persluphate) for a FIA spectrophotometric method for the determination of chlorpromazine in aqueous solutions and pharmaceutical formulations.

Experimental

Chemicals

All chemicals used in this project were of analytical grade reagents unless otherwise stated. Table (1) tabulate the main chemicals used throughout this research work.

Table (1): The main chemical used throughout this research work

Name	Formula	Supplier	Concentration (g/100ml)
Sodium persulphate ($1mole.L^{-1}$)	$Na_2S_2O_8$	BDH	11.9050g
Chlorpromazine HCl (5mM)	$C_{17}H_{20}Cl_2N_2S$	SDI	0.1776g

Gel Bead (Hydrogel)

Gel bead was washed with distilled water. This was repeated with constant change of distilled water between time to time to insure a complete removal of any salt (if present) inside gel bead. No problem was met in introducing the gel bead into gel bead cell housing unit. Figure (2) shows the shape and the geometrical arrangement of the growth of gel beads until it gain a regular spherical shape.

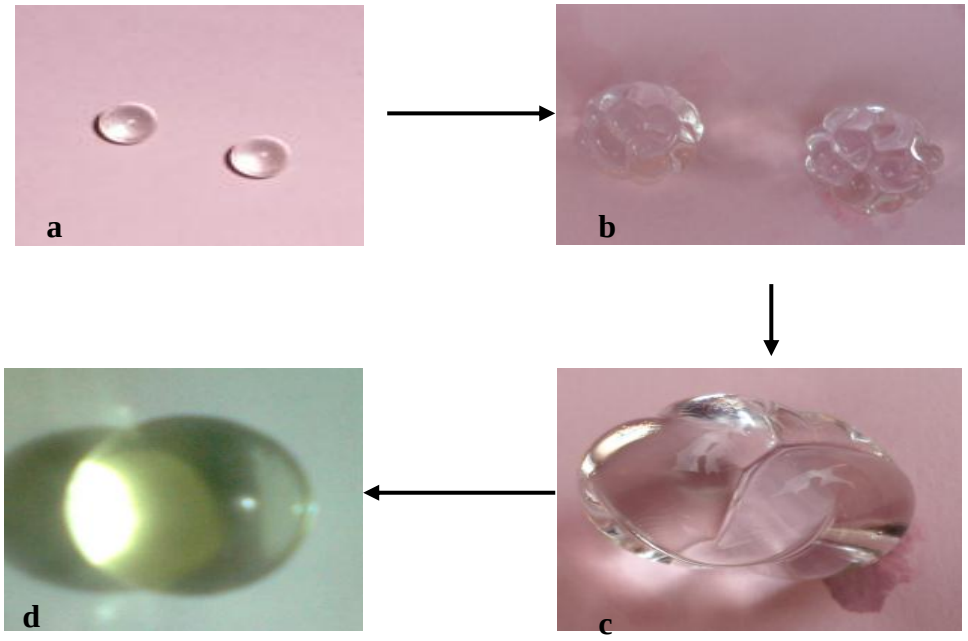


Figure (2): Variable growth time for gel bead when it's soaked in distilled water
(a) Time of zero time (b) Time lapse of 4hours
(c) Time lapse of 8hours (d) Time lapse of 16hours

Apparatus

Gel bead cell housing unit (homemade)

Home-made design of gel bead cell housing unit was designed to accept a swollen gel bead.

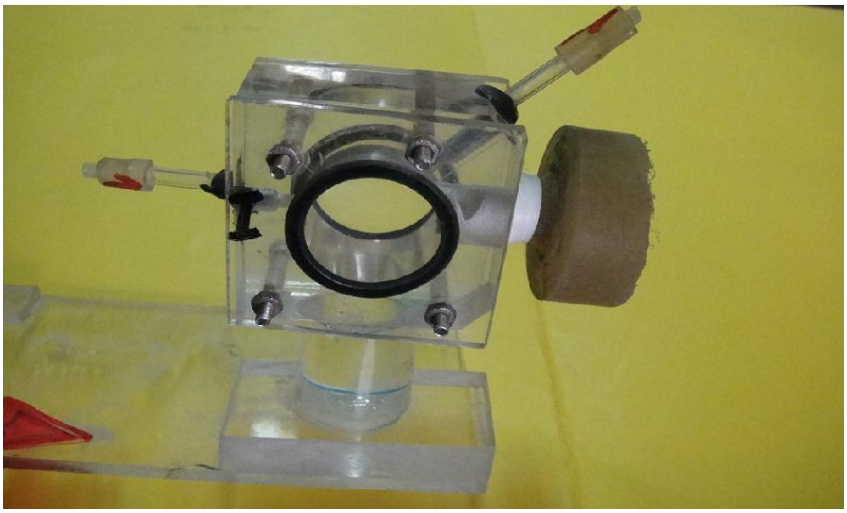


Figure (3): Home-made Gel bead cell housing unit

Methodology and reaction manifold design

The reaction manifold system is very simple and it is composed of single manifold as shown in figure(4); in which distilled water line passes to the gel bead cell unit containing sodium persulphate solution that was entrapped inside gel bead. Due to concentration gradient in sodium persulphate solution between the gel bead (solid phase) and liquid phase, the oxidizing agent solution is released from hydrogel bead and passes by the effect of carrier stream to injection valve (up church scientific INC) where the sample is injected. Colored complex is formed and the variation in response was monitored using home-made Ayah3Sx3-3D solar (Local market) FI photometer and super bright green light emitting diod (local market) as a radiation source throughout this work.

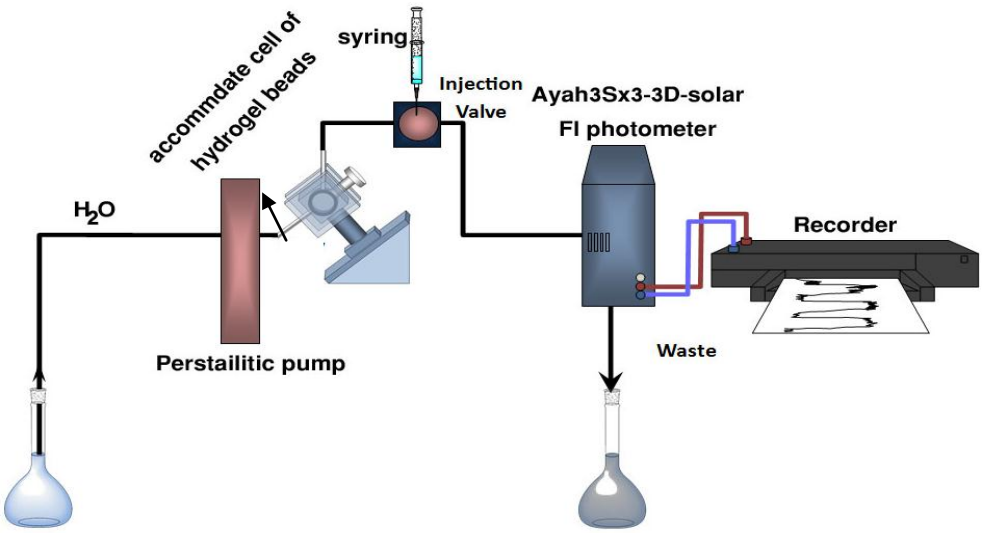


Figure (4): A flow-diagram for the chlorpromazine HCl-$Na_2S_2O_8$ system using gel bead as a host for sodium persulphate solution

Results and discussion

Spectroscopic study

A study was carried out to scan the colored complex that was produced when chlorpromazine HCl is reacted with sodium persulphate to form an intense pinkish-red species. Figure (5) shows the height response of pinkish-red species of newly developed reaction of chlorpromazine HCl-$Na_2S_2O_8$ using Ayah3Sx3-3D solar FI photometer at three different light Emitted Diod (LED) [blue(470nm), green(525nm), and red(635nm)]. A maximum response measured in mV obtained when using the high intensity green light Emitted diod (525nm) as a radiation source. Therefore, the super bright green light emitting diod was used throughout this work.

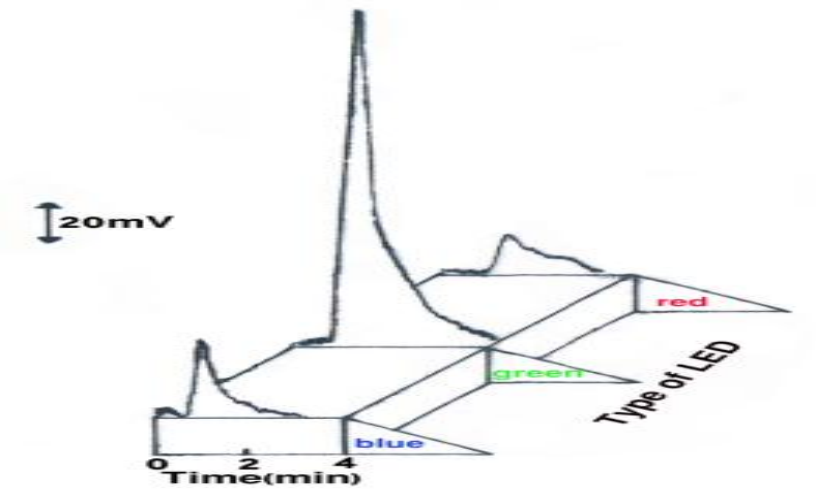


Figure (5): Response-time profile of the colored complex at three different light emitting Diod (LED)[blue(470nm),green (525nm) and red(635nm)] using home-made yah3Sx3-3D-solar FI photometer

Preliminary investigations

A swollen hydrogel bead was immersed into sodium persulphate solution for few hours, followed by transfer to a beaker containing distilled water. Few drops of chlorpromazine HCl was added to the same beaker, an oxidation process was observed followed by the formation of pinkish colored complex indicating that sodium persulphate solution was absorbed and released to the surrounding solution that contains chlorpromazine HCl as shown by the formation of colored complex (fig(6A)). While figure (6B)) illustrate the isolation of the hydrogel bead and the adsorbed oxidation product on it's surface. Therefore, the gel bead can be used as a host for the oxidizing agent solution. Thus developing a new method for the determination of chlorpromazine HCl in pure and pharmaceutical formulations.

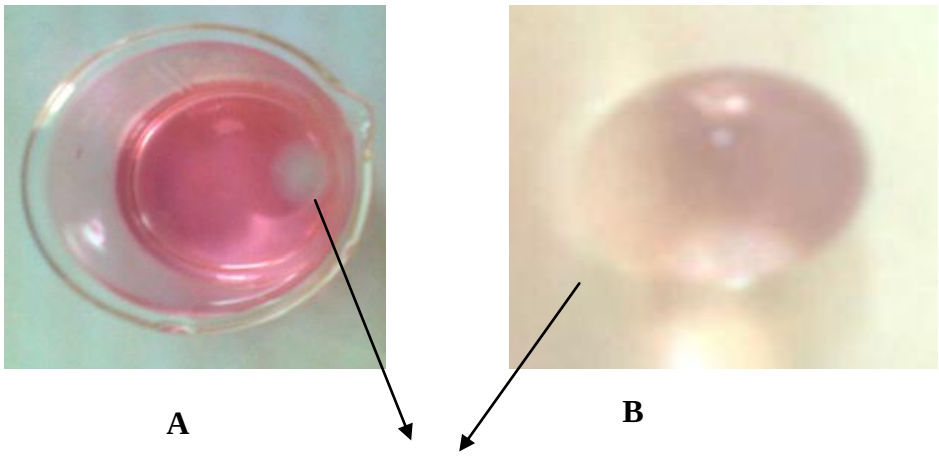


Figure (6): (A) Formation of colored complex due to released $Na_2S_2O_8$ from gel bead and (B) Isolation of gel bead from the colored solution showing the colored product adsorbed on it's surface

Efficiency of hydrogel beads

Using the manifold reaction system as mentioned above, a study was carried out to establish the number of hydrogel bead that is necessary for the entrapment of sodium persulphate. Separately single and two gel beads that previously immersed inside sodium persulphate solution (0.7 mole.L^{-1}) were placed inside gel bead cell housing unit. 100µl of chlorpromazine HCl(0.4 mM) and 1.7ml.min^{-1} flow rate were used. The analysis time when using two hydrogel beads was more than single hydrogel bead as shown in figure(7A) might be due to dispersion in sample segment and because the excessive amount of released oxidizing agent . Therefore, the final decision for the number of gel beads was single hydrogel bead that can be used through out the forth coming experiments.

The number of injections and repeatability for single hydrogel bead were also investigated. An increase in the measured responses was obtained for the first five successive measurements because the difference in oxidizing agent concentration inside and outside the gel bead, followed by a steady-state response within thirty eight injections might be due to equilibrium state, then a decrease in the responses was observed as illustrated in figure (7B). Therefore, all the optimum parameters were studied at the steady-state of measurements in the next coming experiments.

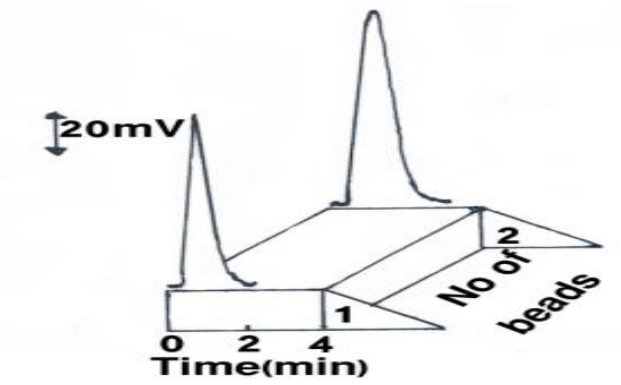


Figure (7A): Response-time profile of variable number of hydrogel beads entrapped inside cell housing unit

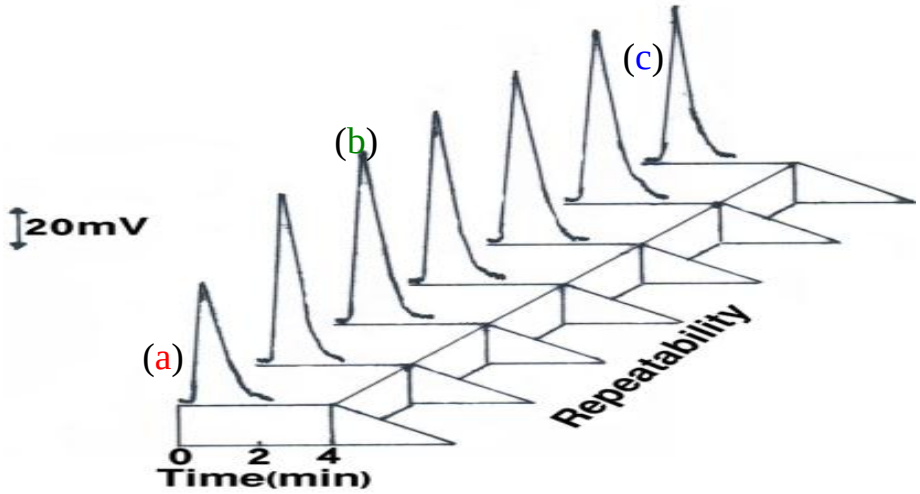


Figure (7B): Response-time profile of chlorpromazine HCl-$Na_2S_2O_8$ system showing a sample of(a) initial, (b) steady-state and (c) end of measurements.

Study of the variables

The physical parameters

A set of experiments were carried out to establish the physical variables through out this work. A study was conducted to optimize the flow rate ranging 0.5-2ml.min^{-1} were used. Using 100µl as injected sample volume of 0.4 mM chlorpromazine HCl, twelve seconds as purge time and single hydrogel bead that was immersed in a previous step inside sodium persluphate solution(0.7mole.L^{-1}) for two and half hours. Figure (8A) shows the kind of response-time profiles. From the obtained results 1ml.min^{-1} gave a higher, more sensitive and lesser base width this might be due at low flow rates there were an increase in peak base width (Δt_B) due to the dispersion and dilution which causes an irregular responses while, at higher speed**(**> 1 ml.min^{-1}), although the effect of physical parameter was very crucial on the response obtaining

regular responses and very sharp maxima, therefore a flow rate of 1ml.min^{-1} was chosen as an optimum flow rate.

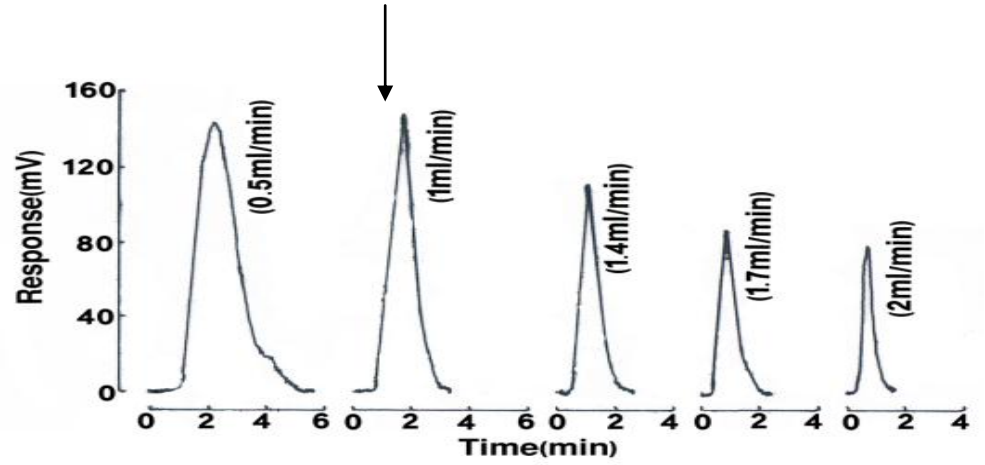


Figure (8A): Response-time profile of variable flow rate using single gel bead

Variable sample volumes ranging from40 to 250µl were also study adopting the same procedure which comprises the use of 1ml.mi^{-1} as flow rate. Table(2) tabulate the obtained results which shows that there is an increase in the responses up to 100µl sample volume, while no gain in the obtained responses was observed above 100µl in spite of the increase in base width of responses(i.e. increase analysis time). Therefore, a compromise was made between the sensitivity and analysis time leading to the use of 100µl as the optimum of volume sample.

Table (2): Variation of responses with sample volumes using gel bead mode

Sample volume(µl)	Response (n=3) Y_i(mV)	Average Response $\acute{Y}_i$(mV)	σ_{n-1}	R.S.D%	Confidence interval of average response(95%) $\acute{Y}_i \pm t_{0.05/2}\ \sigma_{n-1}/\sqrt{n}$
40	50.5,51.5,51.0	51.00	0.50	0.98	51.00±1.24
72	104.0,103.9,104.5	104.10	0.32	0.30	104.10±0.79
100	151.0,150.7,151.8	151.16	0.56	0.37	151.16±1.39
180	152.0,151.0,153.0	151.00	1.00	0.66	151.00±2.48
250	152.0,150.0,149.8	150.60	1.21	0.80	150.60±3.00

The purge time of injected sample segment from injection valve was studied at variable time lapse (3-18seconds) using the achieved parameters. Figure (8B) shows that more than twelve seconds are required. Therefore, fifteen seconds was chosen as the optimum purge time. These studies conclude that 1ml.min^{-1} flow rate, 100µl sample volume and 15seconds as purge time of injected sample volume will be used through out this work.

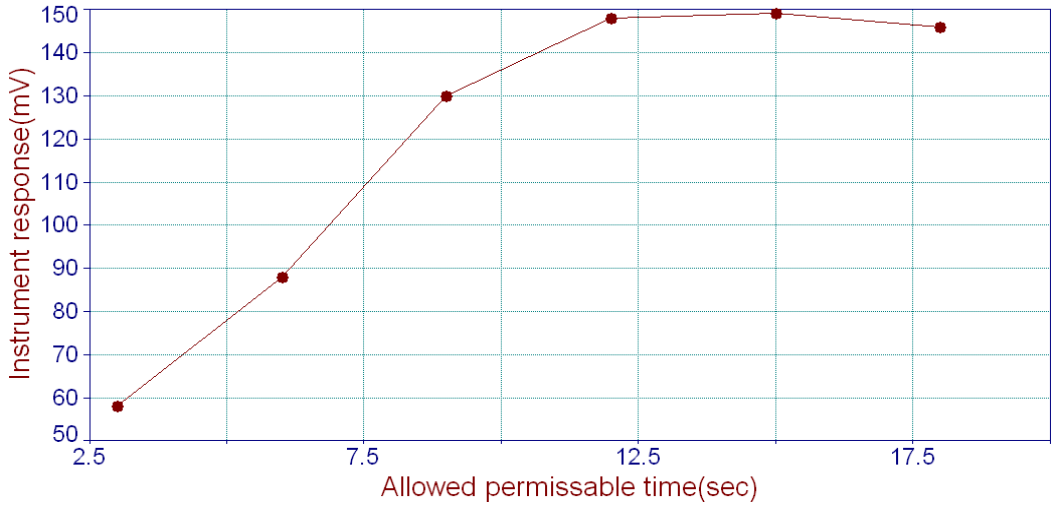


Figure (8B): Effect of variation of allowed permissible time(sec) on the obtained instrument response(mV) using 100µl of 0.4 mM chlorpromazine HCl

The chemical parameters

A series of experiments were carried out to determine the optimum concentration for the sodium persulphate solution in the range (0.1-1.0mole.L^{-1}) using the optimum parameters achieved in previous section taking into consideration that single gel bead was imbedded previously into each of five beakers containing variable concentrations of aqueous sodium persulphate for two and half hours then transferred to gel bead cell unit. Table (3) tabulate the results obtained indicating that 0.5mol.L^{-1} was the most suitable concentration to conduct the oxidation process. Therefore, 0.5mol.L^{-1} was chosen as optimum concentration in the subsequent work.

Table (3): Effect of variation of enclosed sodium persulphate concentration using single gel bead imbedded on the response of colored complex

$[Na_2S_2O_8]$ mol.L^{-1}	Response (n=3) Y_i(mV)	Average response $\acute{Y}_i$(mV)	σ_{n-1}	R.S.D%	Confidence interval of average response(95%) $\acute{Y}_i \pm t_{0.05/2}\ \sigma_{n-1}/\sqrt{n}$
0.1	88.0,88.0,86.0	87.30	1.15	1.31	87.30±2.85
0.3	118.0,118.0,120.0	118.60	1.15	0.96	118.60±2.85
0.5	149.0,147.0,148.0	148.00	1.00	0.67	148.00±2.48
0.7	150.3,150.0,149.6	149.96	0.35	0.23	149.96±0.23
1.0	152.0,152.0,151.0	151.60	0.57	0.37	151.60±1.41

The effect of time that is necessary for the swollen gel bead to be immersed inside sodium persulphate solution before transferring to gel bead cell unit was studied using the manifold reaction system as mentioned before. 1-4hours were used as a soaking

time; table (4) lists the average of three successive measurements, indicating that three hours was the most suitable time giving a constant and enough amount of released sodium persulphate solution for the measurements.

Table (4): Effect of variation of soaking time of single gel bead inside sodium persulphate solution on the response of colored complex

Soaking time(hours)	Response (n=3) Y_i(mV)	Average response $\acute{Y}_i$(mV)	σ_{n-1}	R.S.D%	Confidence interval of average response(95%) $\acute{Y}_i \pm t_{0.05/2}\ \sigma_{n-1}/\sqrt{n}$
1.00	44.0,42.0,42.0	42.6	1.15	2.71	42.6±2.85
1.30	94.0,94.0,94.0	94.0	0.00	0.00	94.0±0.00
2.00	140.0,139.6,140.4	140.0	0.40	0.28	140.0±0.99
2.30	146.0,145.0,147.0	146.0	1.00	0.68	146.0±2.48
3.00	148.0,148.0,148.0	148.0	0.00	0.00	148.0±0.00
4.00	119.0,120.0,118.0	119.0	1.00	0.84	119.0±2.48

Construction of calibration curve

A series of chlorpromazine HCl solutions ranging from0.00 to 2.00mM were prepared using all achieved optimum parameters in previous sections. A scatter plot diagram was carried out between the variations of the obtained responses chlorpromazine HCl concentrations shows a linear dynamic range from 0.17 to 0.80mM. Figure(9) shows the calibration graph using simple linear equation of form y=a+bx, while table(5) tabulate the correlation coefficient(C.C), percentage linearity, straight line equation and the calculated t-value at 95% confidence of 28.71 which larger than tabulated t-value indicating clearly that the linearity against non-linearity is accepted.

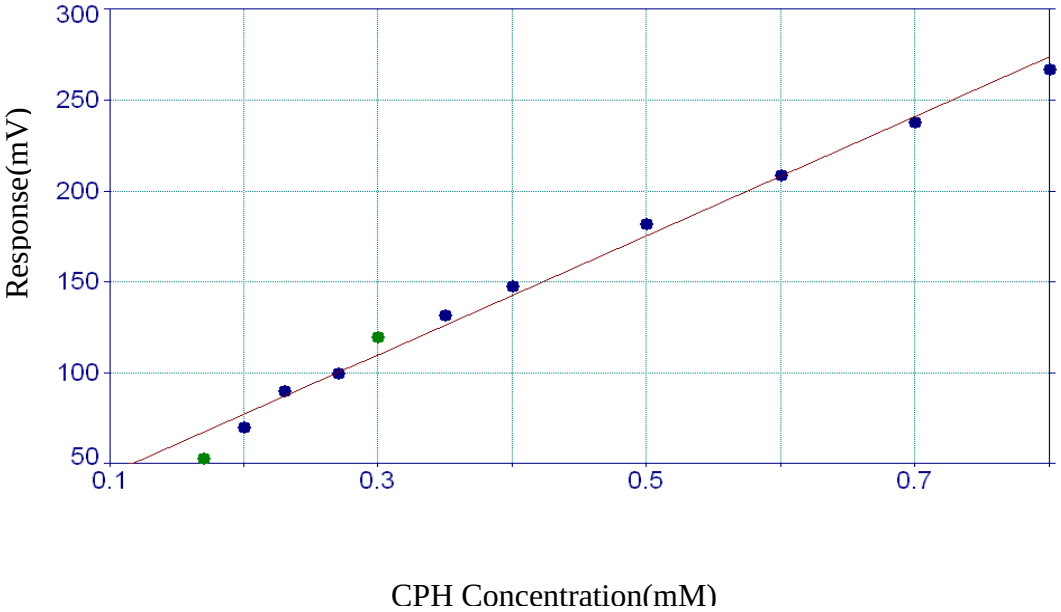


Figure (9): Linear calibration graph for the variation of instrument response versus chlorpromazine HCl concentration using simple linear equation represented as: response(mV)=intercept+slope[chlorpromazine HCl]mM

Table (5): Summary of calibration graph using first degree equation

Concentration (mM)	Linear range (mM)	Straight line equation $\hat{Y}_{mv}=a\pm s_a t+b\pm s_b t[x]$	Correlation coefficient (r)	Percentage Linearity(r^2%)	Calculated t-value $\frac{/r/\sqrt{n-2}}{\sqrt{1-r^2}}$	t-value tabulated at 95% confidence interval(n-2)
0.00-2	0.17-0.8	$\hat{Y}$=11.50±5.21 +327.98±11.39[x]	0.9945	98.92	28.71>>2.262	

Limit of detection (L.O.D)

The limit of detection of adopted developed method for determination of chlorpromazine HCl was measured using two different methods [36]. The practical limit of detection was measured from gradual dilution of minimum concentration in the calibration range (0.17mM). Table (6) tabulates the limit of detection of chlorpromazine HCl using two different methods.

Table (6): The limit of detection of chlorpromazine HCl using the optimum parameters of FIA-gel bead method

Based on gradual dilution of minimum concentration	Based on value of slope $X=3S_B/slope$
$0.108x10^{-9}M$	$6.99x10^{-9}M$

Repeatability

The repeatability of measurements and the efficiency of proposed method was studied using a randomly selected concentration of chlorpromazine HCl(0.5mM). Six successive measurements were used for this experiment (table 7). A value of relative standard deviation of 0.108 indicates clearly the high efficiency of the new adopted method.

Table (7): The repeatability of six successive measurements at the optimum parameters

[chlorpromazine HCl]mM	Number of injection	Average response $\acute{Y}_i$(mV)	σ_{n-1}	R.S.D%	Confidence interval of the average response (95% confidence) $\acute{Y}_i \pm t_{0.05/2}\ \sigma_{n-1}/\sqrt{n}$
0.5	6	184.60	0.20	0.108	184.60±0.209

The application

Chlorpromazine HCl was determined in the pharmaceutical formulation using the new developed method under the optimum parameters achieved in previous sections. The obtained results were compared with quoted value from the manufacturer company (SDI). Thirteen tablets were weighed, crushed and grinded to a fine powder (dusty); 2.5mM of chlorpromazine HCl was prepared as stock solution and 1.00ml was pipetted to each of five 25ml volumetric flasks followed by the addition of gradual volumes of standard chlorpromazine HCl (0.0, 1.7, 3.4, 5.1, 6.8ml) of 2.5mM to obtain 0.1, 0.27, 0.44, 0.61, 0.78mM, where the first flask contains zero added standard chlorpromazine HCl. The obtained results were tabulate in table (8), while figure (10) shows the calibration graph of standard addition method of chlorpromazine HCl.

Table (8): The obtained results for determination of chlorpromazine HCl using gel bead as a supplier for sodium persulphate solution

Commercial name, Content & country	Confidence interval for the average weight (95%) $\acute{w}\pm1.96\ \sigma_{n-1}/\sqrt{n}$	Sample weight(0.8882mg) equivalent to 0.1mmole.L^{-1} of active ingredient(g)	Theoretical content of active ingredient at 95% n=∞ (mg)	Practical content of active ingredient at 95% n=∞ (mg)	Recovery%
Largcitel 50mg SDI Iraq	0.3596±0.0102	0.0065	50±0.0014	50.12±0.78	100.24

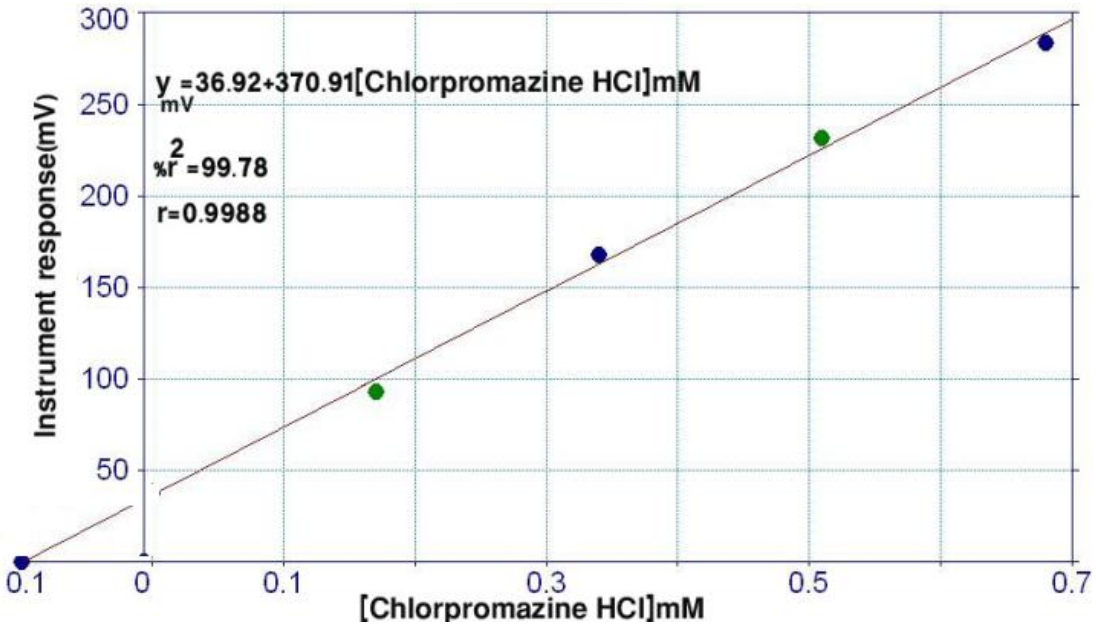


Figure (10): The calibration graph of standard addition method for determination of chlorpromazine HCl using gel bead as a host for sodium persulphate solution

Paired t-test was used to compare the efficiency of the new developed method for determination of chlorpromazine HCl with the quoted value at 95% confidence interval. The obtained results were tabulate in table(8) indicate that there was no significant difference between the new method and claimed method by the company as calculated t-value is less than tabulated t-value. On this base the new method can be used as an alternative analysis method for the determination of chlorpromazine HCl in pharmaceutical formulation.

Table (8): Paired t-test for the FIA-gel bead method with quoted value for determination of chlorpromazine HCl

No of measurements	Practical content(mg)		d (mg)	$\bar{X}_d$	σ_{n-1}	Paired t-test $\bar{X}_d\sqrt{n}/\sigma_{n-1}$	t_{tab} at 95% confidence interval,n-1
	Proposed method	Quoted value					
1	50.12	50	0.12	0.44	0.639	1.19<<4.303	
2	50.03	50	0.03				
3	51.18	50	1.18				

Conclusions

It could be concluded that the developed method for chlorpromazine assay is simple, sensitive, relatively precise, and accurate and can be satisfactorily applied to the analysis of chlorpromazine in bulk and pharmaceutical formulations. The proposed method was used for the routine analysis of the drug in the quality control.

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