New Approach for the online determination of Paracetamol using merging zone-continuous flow injection analysis via home made photometric based on 704 nm LED and a photo silicon minidetector

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Abstract

A simple, rapid and sensitive spectrophotometric method for the determination of paracetamol using merging zone-continuous flow injection analysis technique via hexagonal flow cell and home made photometric based on 704 nm LED and a photo silicon minidetector was studied. The method was based on the oxidation of paracetamol by Fe(III) solution which leads to the formation of Fe(II) which in turn , reacts with Potassium hexacyanoferrate(III) to form Prussian blue dye and determination of this dye at 704 nm.Chemical and physical parameters of this system were investigated. The analytical graph is ranged from (0.0 – 10 mmol.L^{-1}) with a limit of detection 2.0 nmol.L^{-1}.The relative standard deviation was 0.08% for 6.0 mmol.L^{-1} paracetamol solution (n=8).The method was applied for the determination of paracetamol in pharmaceutical formulations.

الخلاصة

تم تقدير عقار الباراسيتامل باستخدام طريقة طيفية ضوئية بسيطة و سريعة وحساسة في المستحضرات الصيدلانية باستخدام تقنية اندماج المناطق – التحليل بالحقن الجرياني المستمر عن طريق خلية جريان عابر سداسية الأسطح وباستخدام مصدر مطياف ضوئي ذو طول موجي ٧٠٤ نانومتر مصنع محليا وبعد ذلك تم كشف الضوء الممتص من قبل متحسس مايكروي سيليكوني ضوئي.ان طريقة التقدير تعتمد على أكسدة عقار الباراسيتامول بواسطة ايون الحديد الثلاثي (Fe(III)) مؤدياً الى تكوين ايون الحديد الثنائي (Fe(II)) الذي بدوره يتفاعل مع بوتاسيوم سداسي سيانيد الحديديك K_4 [$Fe(CN)_6$] لتكوين الصبغة البروسية الزرقاء وقد م قد تم تقدير هذه الصبغة في ٧٠٤ نانومتر.تم دراسة كل المتغيرات الفيزياوية والكيمياوية للحصول على أفضل النتائج. منحنى المعايرة يخضع الى قانون بير– لامبرت ضمن المدى (٠,٠ – ١٠) ملي مول. لتر $^{-١}$ و بحد كشف مقداره (٢,٠) نانو مول. لتر $^{-١}$.الانحراف القياسي النسبي لـ ٦,٠ ملي مول.لتر $^{-١}$ (ن=٨) كان ٠,٠٨%
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Introduction

Acetaminophen is acetamide, N-(4-hydroxy phenol)-N,N-acetyl-P-amino phenol; which is prepared by reduction of p-nitrophenol and the resulting p-aminophenol is acetylated by heating with a mixture of acetic anhydride and glacial acetic acid. The crude product may be purified by recrystalization for ethanol-water mixture [(1)]. It's effective in a wide variety of arithmetic and rumatic conditions involving muscular skeletal pain as well as the pain of headache, dysmenart, myalgas and neuralgias. Acetaminophen is particularly useful as an analgesic – antipyretic in patients sensitive to aspirin, and who experience other untoward reaction to aspirin[(1)]. Different spectrophotometric methods were used for the determination of paracetamol whether alone or in admixture, one of the recent publications was the determination of paracetamol and caffeine based on the difference in the rate of oxidation with Cu(II) –neocuprion system and formation of Cu(I) – neocuprion which was monitored at 453 nm at PH 5.0 in the presence of Sodium Dodesyle Sulphate (SDS) , analytical range 1.5 - 6.0 µg.mL^{-1} can be determined[(2)]. Also the possibility of the determination of Paracetamol based on the catalytic effect of Mn(II) on the oxidation of Paracetamol by Cr(VI) in the presence of perchloric acid was studied kinetically but nothing was mentioned about linear range , Limit of detection and any other parameters[(3)]. Paracetamol was determined via reduction of Fe (III) ion and complexation with 1, 12-phenanthroline at specified PH condition that enabled 0.5 – 10 µg.mL^{-1} to be determined [(4)] .Sodium hypochlorite was used for the determination of paracetamol through its oxidation and determining the excess of oxidant by reaction with o-tolidine dichloride as chromogenic reagent and measurement of absorbance at 430 nm using flow injection analysis (FIA). The method claimed to be able to determine 8.5 x 10^{-6} – 2.5 x 10^{-4} mol.L^{-1} at LOD 5x 10^{-6} [(5)]. Sequential flow injection analysis using spectrophotometry was used to the determination of paracetamol with linear range 6.6 x 10^{-5} –1.32 x 10^{-3} mol.L^{-1} at 60 sec measurement time using oxidation of paracetamol with potassium permanganate in sulphuric acid medium [(6)].Second derivative spectrophotometric method was used for the determination of paracetamol with 2, 2'-bipyridyl as a complexing agent and measuring the absorbance at 522 nm. Linear dynamic range was from 0.0 – 12 µg.mL^{-1}[(7)]. Prussian Blue (PB) is obtained by the addition of Fe(III) salt to a solution of $[Fe(CN)_6]^{4-}$ .Turn bulls blue (TB) is formed by the addition of Fe(II) salt to a solution of $[Fe(CN)_6]^{3-}$. It's known appreciated that TB and PB are the same because of the rapidity of electron exchange through a Fe-CN-Fe linkage. The exact hue depends on the method of preparation which dictate the energy of the transfer of electrons from Fe(II) to Fe(III) [(8)] .The work conducted in this research relies on the use of p-acetaminophen (paracetamol) as a reductant for the prepared Fe(III) ion to form Fe(II) using merging zone utilizing two loops(L_1 , L_2) then allow to complete the reaction in L_3, then the formed Fe(II) ion meets $[Fe(CN)_6]^{3-}$ to form turn bulls blue (TB) or Prussian blue(PB) which is allowed to react and complete the reaction within 30 sec in loop 4 then passes through flow cell where it irradiated with 704 nm LED at variable intensity using photo silicon diode as a detector (reference is made to fig.1).

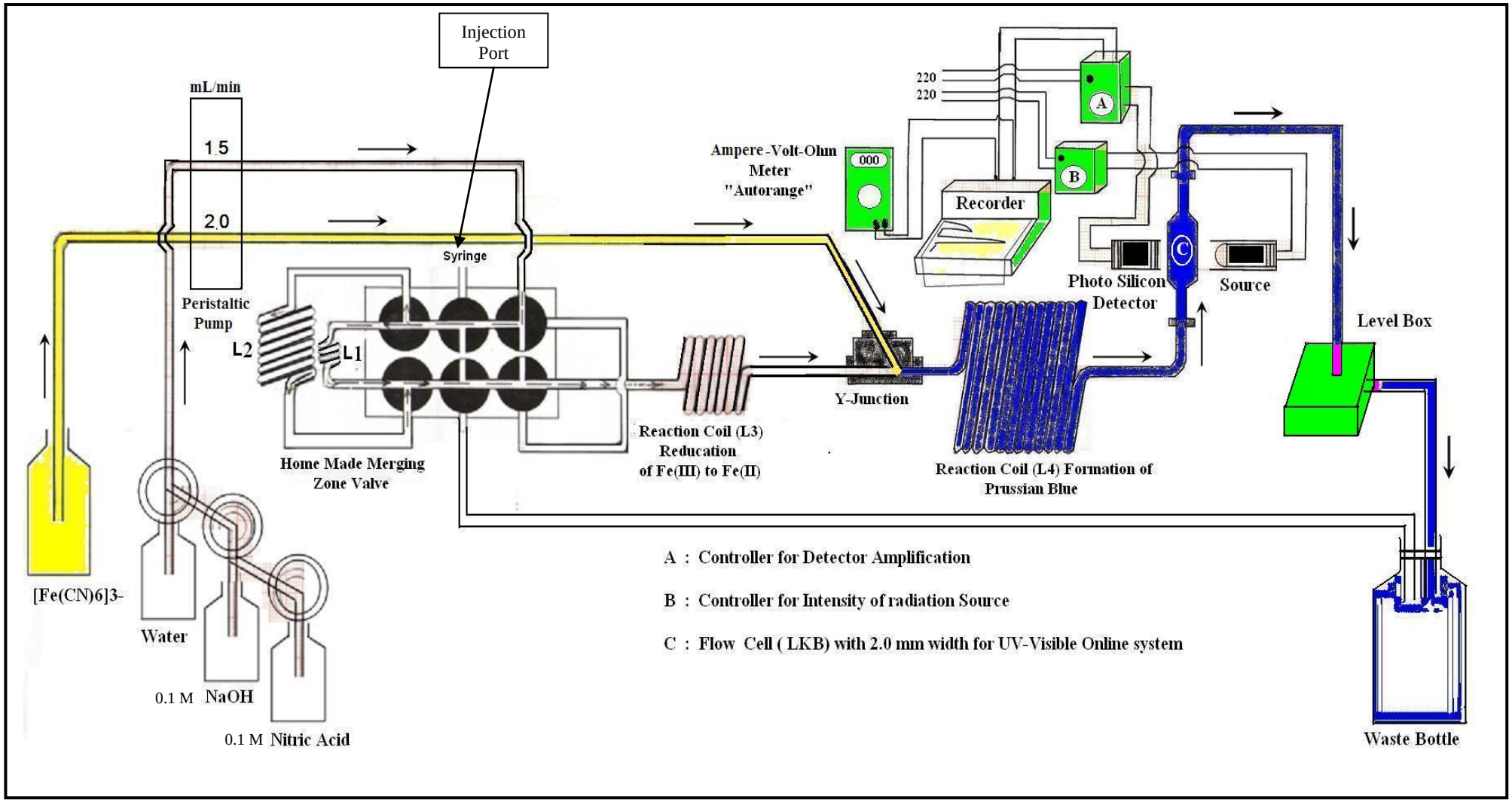

Fig 1: Flow diagram manifold system used for the determination of Paracetamol

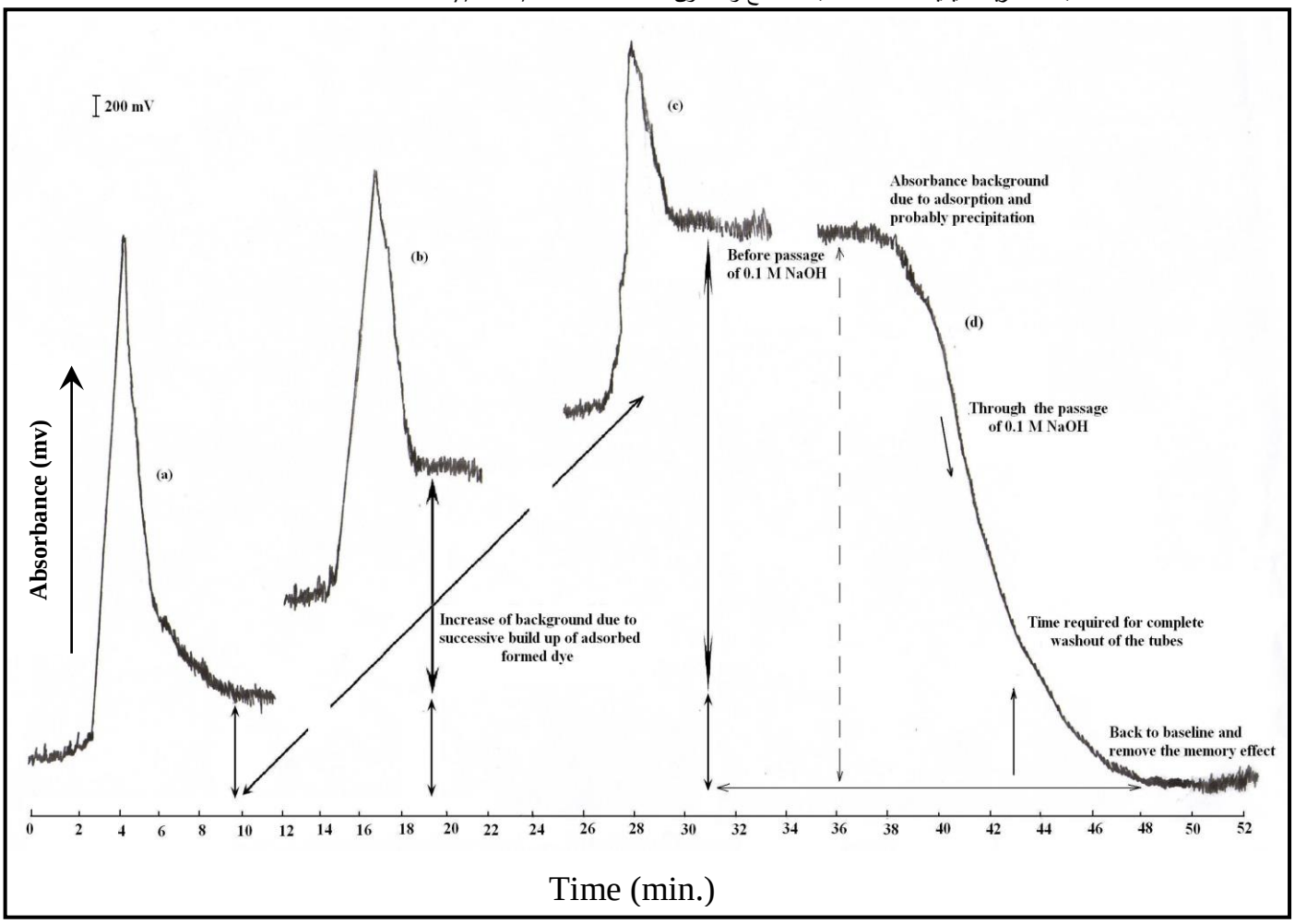

Fig 2: Response – Time Profile for the determination of Paracetamol using $[Fe(CN)_6]^{3-}$ (8.0 mM.L^{-1}) - Paracetamol (5.0 mM.L^{-1}) – Fe(III) (5.0 mM.L^{-1}) system.

Methodology

Following the flow diagram shown in Fig 1., loop1 is loaded with p-acetaminophen (40µL) ,loop 2 is loaded with Fe(III) ion (100µL),loop3 is a delay reaction coil for completion of reaction and reduction of Fe(III) to Fe(II) and loop 4 is reaction coil for completion of reaction and formation of Prussian blue. Carrier stream flow rate is 1.5 mL.min^{-1} while $[Fe(CN)_6]^{3-}$ flow rate was 2.0 mL.min^{-1}. The time taken from the departure of the merged zone from valves to the measuring cell is 60 sec, followed by the washing with NaOH 0.1 M passed through $[Fe(CN)_6]^{3-}$ line until read zero background because different concentrations of formed dye will require different washing time. Fig 2 shows three successive measurements (a,b,c) and response obtained which clearly indicate the memory effect formed by the adsorption of the blue dye on the glass wall. while in the same figure (d) ,it shows washing with 0.1 M NaOH solution and It also shows the return back to the background level, then followed washing with 0.1 M HNO_3 at the end of working day. This treatment is quite necessary for good repeatability and reproducibility measurements, due to high adsorbing effect of formed dye.

Experimental

Chemicals

All experiments were performed with chemicals of analytical grade reagents:

- Paracetamol 0.1 mol.L^{-1} (BDH, UK) was prepared by dissolving 3.779g in 250 ml distilled water.
- Iron(III) 0.1 mol.L^{-1} (FLUKA , Germany) was prepared by dissolving 13.901g $FeSO_4.7H_2O$ followed by the addition of 2-3 drops of concentrated sulfuric acid after dissolution in little amounts of water(approximately 200mL followed by the addition of 90 mL of hydrogen peroxide 0.1 mol.L^{-1} which is prepared and purified through passing on a cation exchanger Amberlite 120) warming the solution with constant stirring for the completion of oxidation of Fe(II) into Fe(III), continuation of doing so until bubbles ceases in order to get rid of excess of H_2O_2 followed by the addition 10-15 mL of concentrated H_2SO_4 in order to avoid the turbidity of the solution during the process of oxidation of Fe(II) to Fe(III).Quantitatively the solution transferred into a volumetric flask (500mL) ,the volume is completed with distilled water.
- Potassium hexacyanoferrate(III) $K_3[Fe(CN)_6]$ 0.1mol.L^{-1} (BDH , UK) was prepared by dissolving 16.463g in 500mL.Also 0.1 mol.L^{-1} NaOH and 0.1 mol.L^{-1} HNO_3 each were also prepared.

Apparatus

- Schematically Fig 1 shows different parts used in the manifold system. The valve[9], the detector, the source and the amplification unit are completely home made
- Loops , (L1 , L2) are made of Teflon (1.5 mm i.d) while (L3 , L4) are made of glass
- Y-Junction are made of Teflon and Glass
- Peristaltic Pump (Ismatec , Switzerland) ,4 – Channel
- Graph x-t Chart recorder (C/032)(SIEMENS , Germany)

Results and Discussions

Using preliminary experimental concentrations of the chemicals used in the main reaction where as following: Fe(III) 5.0 mmol.L^{-1} , paracetamol 5.0 mmol.L^{-1} and $K_3[Fe(CN)_6$ 8.0 mmol.L^{-1} .Fig 3(a,b,c) shows the absorbance spectra for each of the above prepared solution. The spectrum shows that there is no spectral interferences between the formed complex (broadband, λmax 704 nm) while the remaining two solution (a,b) show λ_{max} 246 nm and 426 nm for paracetamol and hexacyanoferrate(III) solution , respectively.

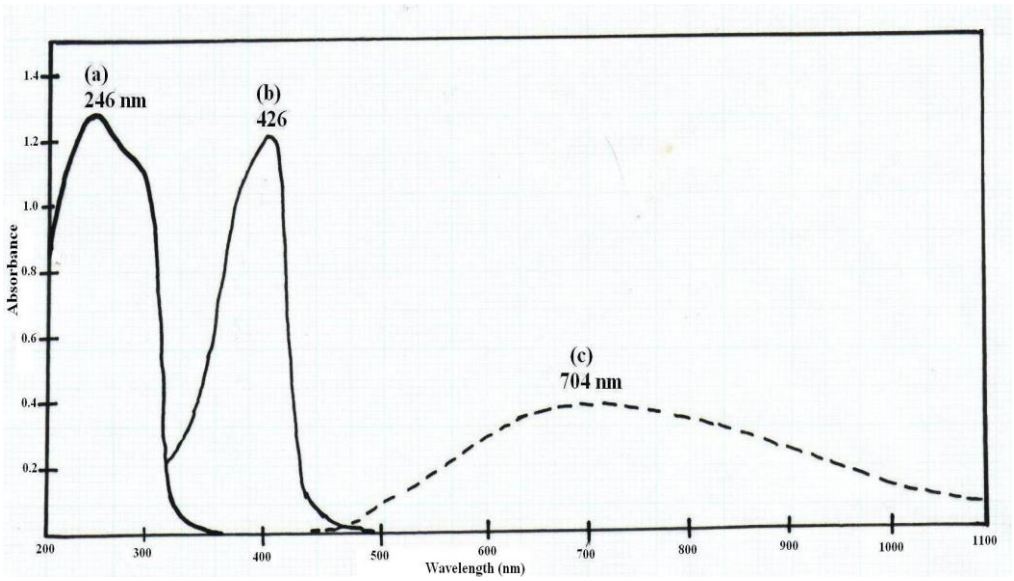

Fig 3: Absorbance spectra for the determination of paracetamol using $[Fe(CN)_6]^{3-}$ - paracetamol - Fe(III)] system

Chemical Variables

A- Variation of Potassium Hexacyanoferrite concentration on the absorbance

Using variable concentration of potassium hexacyanoferrate(III) 0.0 – 15 mmol.L^{-1} and a constant concentration of Fe (III) 8.0 mmol.L^{-1} for the carrier stream. Fig 4 shows these effect which also shows that 8.0 mmol.L^{-1} of potassium hexacyanoferrate(III) is the optimum concentration .At high concentrations, the response are irregular and unclear peak height obtained. It really may be due to increase of the density of the colored product which work as an internal filter that really prevent the remaining light intensity after the absorption process by the colored species to the photo silicon detector. The distortion of the peak maximum might be due to the formation of small colored colloidal precipitate that might formed in front of the detector causing non-return to the background , also the coloration of the tubes with deep blue color.

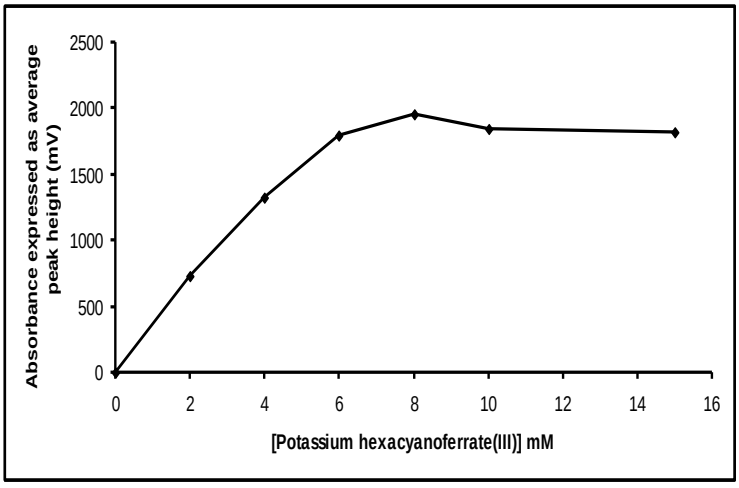

Fig 4: Variation of potassium hexacyanoferrate(III) concentration on the absorbance of Prussian blue complex for the determination of paracetamol

B-Variation of Fe (III) concentration

Series 0.0 – 20 mmol.L^{-1} of Fe (III) were prepared at optimum constant concentration of potassium hexacyanoferrate(III) of 8.00 mmol.L^{-1} and using a preliminary concentration of paracetamol 30 mmol.L^{-1}.Fig 5 shows that 10 mmol.L^{-1} of Fe (III) is a suitable optimum concentration while at a concentration above this; distortion of the peak is observed due to irregulation of response due to formation of tiny blue particles.

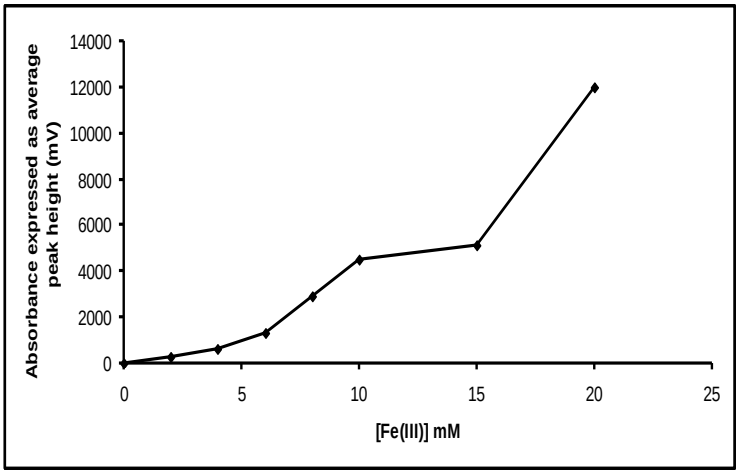

Fig 5: Variation of absorbance versus Fe (III) ion concentration on the formation of Prussian blue.

Physical Parameters

Effect of Flow Rate

Using optimum concentration of the reactant ,Fe(III) 10 mmol.L^{-1} , $K_3[Fe(CN)_6]$ 8.0 mmol.L^{-1} and using a chosen concentration 8.0 mm.L^{-1} of paracetamol using variable 0.7 – 4 mL.min^{-1} flow rate for $K_3[Fe(CN)_6]$ and 0.5 – 3,5 mL.min^{-1} for the carrier stream. Fig 6(a,b) shows that the best flow rate for the completion of the reduction of Fe (III) by paracetamol is 1.5 mL.min^{-1} for the carrier stream and 2.0 mL.min^{-1} for the $K_3[Fe(CN)_6]$.The time required from the moment of departure of the sample from the injection valve till the measuring cell is 60 sec.This time can regarded as the sampling rate.

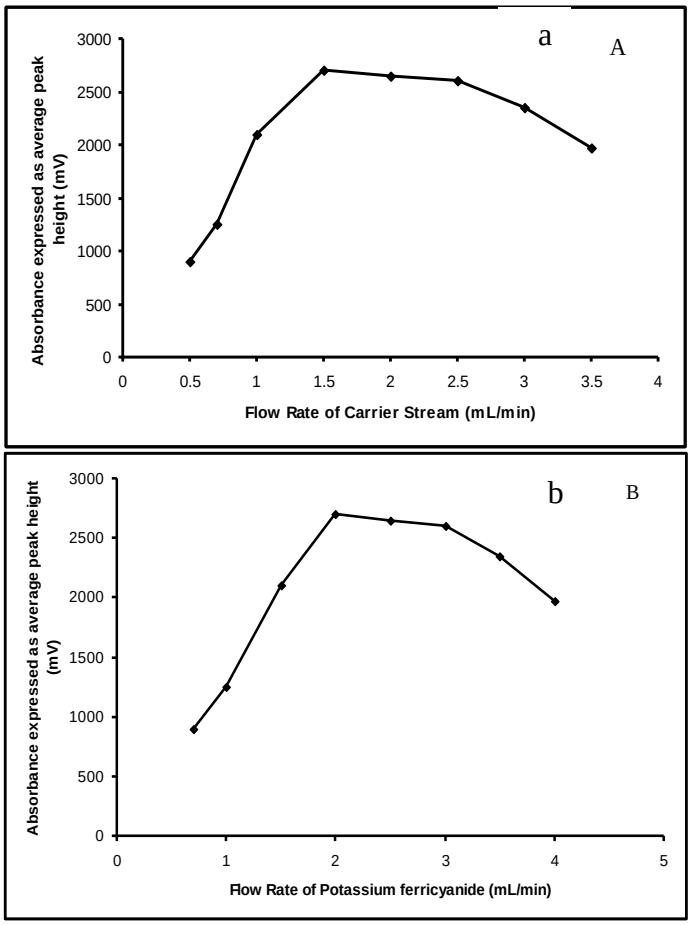

Fig 6: Variation of flow rate of (a) Carrier stream and (b) Potassium hexacyanoferrate(III) on the absorbance of Prussian blue

Variation of Response vs. Paracetamol Concentration

A series 0.0 – 20 mmol.L^{-1} of paracetamol solutions using the optimum concentration arrived to in the previous section. The absorbance was measured at 704 nm.Table 1(a) shows the summary for the variation of response with concentration using linear regression treatment for the range of 0.0 – 10 mmol.L^{-1} while table 1(b) shows the summary for the quadratic regression treatment for the range of 0.0 – 10 mmol.L^{-1}. Fig 7 shows these plots. Quadratic equation shows coefficient of determination (COD) of 98% for the linear plot while it shows 99.9% for the quadratic presentation. Table 2(a,b) shows the ANOVA[10] summary for the linear equation and the quadratic equation which shows that quadratic equation is represented more favorable than linear plot (reference is made to the F-test).

Table 1b: The variation of absorbance with concentration of paracetamol (mmol.L^{-1}) using quadratic regression treatment[10].

Linear Dynamic Range mM.L^{-1}	$Y=a+bX+cX^2$ at Confidence Limit %95 For n-2 $\hat{Y}(mV)=a\pm S_a t+b\pm S_b t[X]+c\pm S_c t[X]^2$	r & r^2%	t_{cal}	t_{tab}	$S_E=(1-r^2)/\sqrt{n}$	$r\pm S_E$
0.00 – 10	$7.96\pm$٩٧.79+180.29±66.5[X]+16.77±6.92$[X]^2$	0.9983 99.66%	48.42>>2.306		0.0058	0.9908±0.0058

Table 1a: The Variation of absorbance with concentration of paracetamol (mmol.L^{-1}) using linear regression treatment[10]

Range of Paracetamol Concentration (Measured) mM.L^{-1}	Linear Dynamic Range mM.L^{-1}	Y=a+bX at Confidence Limit 95% For n-2 $\hat{Y}(mV)=a\pm S_a t+b\pm S_b t[X]$	r & r^2%	t_{cal}	t_{tab}	$S_E=(1-r^2)/\sqrt{n}$	$r\pm S_E$
0.00 - 20	0.00 – 10	125.16±176.65+355.8±37.48[X]	0.9908 98.16%	20.65>>2.306		0.0058	0.9908±0.0058

Table 2a: The analysis of variance ANOVA for linear regression treatment [10]

Source	**Sum of Squares (SSq)**	**df**	**Mean Square (MSq)**	**$F_{statistic}=S_1^2/S_o^2$**
Regression	13130710	1	13130710 30771.721	426.71
Error	246173.77	n-2=8		
Total	13376884	n-1=9		

Table 2b: The analysis of variance ANOVA for quadratic regression treatment [10]

Source	Sum of Squares (SSq)	Df	Mean Square (MSq)	$F_{statistic}=S_1^2 / S_o^2$
Regression	13331731	2	6665865.4	1033.41
Error	45152.709	7	6450.3869	
Total	13376884	9		

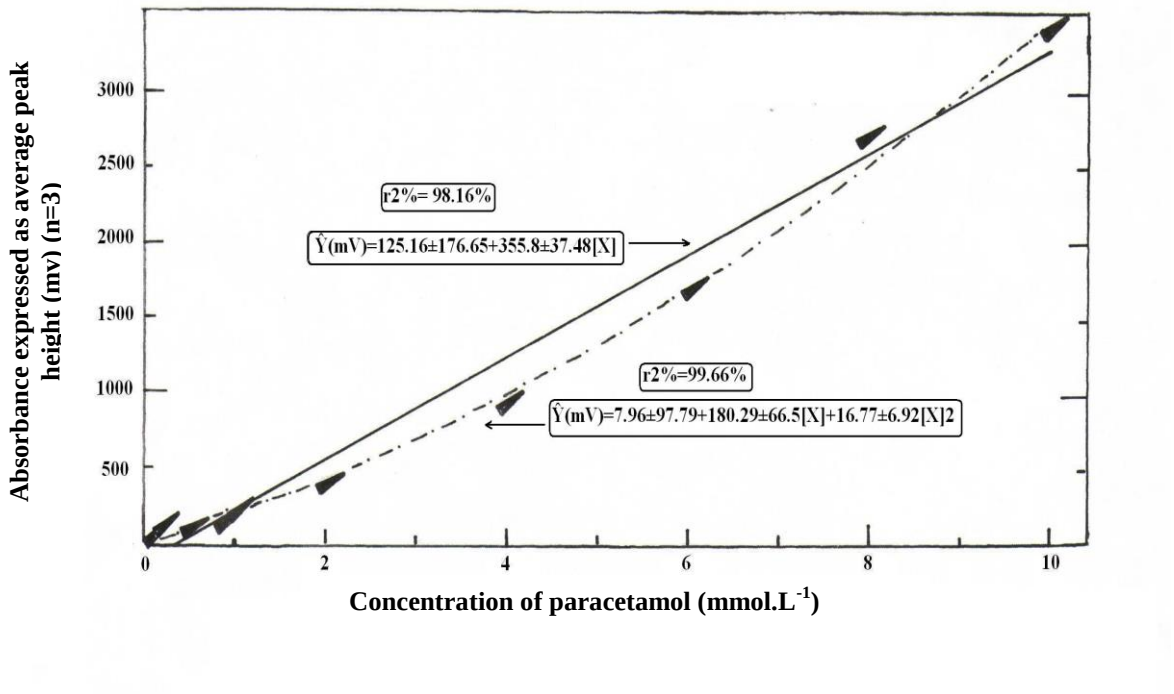

Fig 7: Linear (dark line) and Quadratic (dotted line) plot for the variation of response with concentrations of paracetamol

Detection Limit

Detection limit (DL) [10] is calculated from the gradual dilution of the minimum concentration of the used calibration graph 10^{-4} mol L^{-1}.Table 3 summarizes the four different ways of calculating the limit of detection for the system $K_3[Fe(CN)_6]$ – paracetamol – Fe(III).The volume of the sample used was 40 µL.

Table 3: Detection limit for the determination of paracetamol using $K_3[Fe(CN)_6]$ – paracetamol – Fe(III) system.

Injected Sample Volume (40µL)			
DL from Calibration Graph	$DL=Y=Y_\beta+3S_\beta$	$DL=3S_\beta/Slope$	DL based on dilution factor
2 nM	62.7 nM	0.664 pM	9.52 nM

Repeatability

The work has been conducted in this project was characterized by high precision with good repeatability since the relative standard deviation (RSD) was very small. Table 4 shows the repeatability for the result obtained for the variable concentrations of paracetamol.

Table 4: Repeatability for the determination of paracetamol using $K_3[Fe(CN)_6]$ – paracetamol – Fe(III) system.

[Paracetamol] $mM.L^{-1}$	No. of Readings	Mean ($\dot{y}_i$)	Standard Deviation(σ_{n-1})	%RSD	$t_{0.025,(n-1)}$	Confidence Limit at %95 for n-1 $\dot{y}_i \pm t_{0.025,(n-1)}(\sigma_{n-1}/\sqrt{n})$
0.9	4	١٧٦	0.246	0.1397	3.18	176±0.39
2	٦	٣٩٦	1.367	0.3452	2.57	396±1.43
6	٨	٢٦٩٠	2.098	0.0779	2.36	2690±1.75

Determination of paracetamol in commercially available pharmaceutical tablets

Four different well known drug companies that produce Paracetamol in different formulations where used in this study. For each product of the four selected drug companies, s sample of ten tablets were used for each product in which the individual tablet weight was used to find the average weight for ten tablets, standard deviation and the mean of the average. The mean weight of those tablets is tabulated in table 5.

The ten tablets were grinded, mixed well followed by weighing 0.1 g which was dissolved and filtered (Whatman No.40), in 100mL -volumetric flask and complete to the mark. Using the method established in this work with all parameters involved. Table 6 tabulates the ANOVA for all analyzed samples. It shows that there is at least one of the means is different; therefore, doing Scheffe and Tukey tests[10] to find where the exact difference lies. The results of Scheffe and Tukey tests are tabulated in table 7.

Table 5: Determination of paracetamol in pharmaceutical formulations for four different companies and treatment of data statistically [10]

Pharmaceutical Formulation (n=10)	Components	Weight of tablets(g) $\hat{w}\pm t_{0.05,}\ \alpha\ (\sigma_{n-1}/\sqrt{n})$ $\hat{w}\pm t_{0.01,}\ \alpha\ (\sigma_{n-1}/\sqrt{n})$	Based on Theoretical content (mg)	Practical content(mg)	% Efficiency	$t_{cal}=(\overline{X}-\mu)/(s/\sqrt{n})$ $t_{tab}=4.303$
Paracetamol (SDI – Iraq)	Paracetamol (500mg)	0.6245±0.00303 0.6245± 0.00399	500±2.34 500±3.19	495.79±2.6 495.79±3.43	99.16%	3.17
Dr.Dol (Egypt)	Paracetamol (500mg)	0.5946±0.0049 0.5946±0.0064	500±4.12 500±5.38	493.5±3.98 493.5±5.24	98.7%	3.19
Kanagesic (Damascus-Syria)	Orphenadrine Citrate (35 mg) Paracetamol (450 mg)	0.5327±0.0079 0.5327±0.0104	450±7.42 450±9.76	443±3.99 443±5.26	98.44%	3.43
Relief (Lundic ,India)	Diclofenac Sod. (50 mg) Paracetamol (500 mg) Chloropheniramine Mal. (4mg) Magnesium Trisilicate (100mg)	0.8172±0.0086 0.8172±±0.011	500±5.31 500±6.73	446±3.91 446±5.15	89.2%	27.08

Table 6: The analysis of variance ANOVA [10] for all paracetamol samples.

Source	**Sum of Squares**	**df**	**MSq**	**$F_{cal}= S_B^2/ S_W^2$**	**$F_{0.95;2,9}$**
Between	2.48	K-1=2	$S_B^2 = 1.24$	127.8	4.26
Within	0.0873	N-K=9	$S_W^2 =0.0097$		

Table 7: Treatment of data for paracetamol samples using Scheffe and Tukey tests[10]

Test			F_{tab}	F_{cal}
Scheffe	Paracetamol ($X\acute{o}_1$) Dr.Dol ($X\acute{o}_2$) Relief ($X\acute{o}_3$)	($X\acute{o}_1$)- ($X\acute{o}_2$)	8.52	0.021
		($X\acute{o}_1$)- ($X\acute{o}_3$)		194
		($X\acute{o}_2$)- ($X\acute{o}_3$)		190.02
			F_{tab}	F_{cal}
Tukey	Paracetamol ($X\acute{o}_1$) Dr.Dol ($X\acute{o}_2$) Relief ($X\acute{o}_3$)	($X\acute{o}_1$)- ($X\acute{o}_2$)	3.95	0.203
		($X\acute{o}_1$)- ($X\acute{o}_3$)		19.69
		($X\acute{o}_2$)- ($X\acute{o}_3$)		19.49

*Kanagesic drug was not included in this study due to unsimilarities (450 mg) of basic content of paracetamol

Conclusions

The work presented in this research shows the capability of accepting an alternative method for the analysis and determination of paracetamol using the formation of turn bulls blue (Prussian blue) with good repeatability and an accepting linear dynamic range. The treatment for the data shows that possibility of using quadratic equation parallel with the linear equation. ANOVA analysis shows that the null hypothesis is rejected and the means are not equivalent and for at least one of them. Doing Scheffe test illustrates clearly that there is a difference between tablets manufactured under the commercial label Relief (India) and both Samara drug(Iraq) and Dr.Dol (Egyptian) while there is no difference between Samara drug (Iraq) and Dr.Dol (Egyptian) while Tukey test confirms the above findings which indicate that the drug produced by Lunidic (India) under the commercial name of Relief is different significantly from both other two companies (the high value of F) and this may be attributed to the other components available in the Relief drug.

References

1. Alfonso.R.Gennaro, Remington's Pharmaceutical Sciences , Mack Publishing Company , 17th edition , 1985.

2. H.Tavallali and M.Sheikhaei , ***African J. of Pure and Applied Chemistry***, 2009, **3(1)** , 011-019.

3. M.Llyas , M.A. Malik, S.M.Z.Andrabi and Z.Khan , ***Research Letters in Physical Chemistry*** ,

Vol.2007, Article ID 82901,(2007) 5 Pages.

4. A.Afkhami,N.Sarlak and Ali R. Zarei, ***Acta Chim.Slov***., 2006**, 53,** 357-362.

5. O.Fatibello and H.J.Vieira , ***Ecletica Quimica***, 2008, **33** , 47-53.

6. A.M.Idris , S.M.Sultan , K.E.E.Ibrahem and F.N.Assubaie , ***J.of Flow Injection Analysis*** , 2005, **22(2)** , 123-128.

7. D.T.Burns,N.Tungkananuruk,S.Kas emsumran and K.Tungkananuruk,***Chem. Anal.***(Warsaw), 2005, **50**, 475.

8. Ludi A., "Prussian Blue, an Inorganic Evergreen", ***Journal of Chemical Education***, 1981, **58**, 1013.

9. Issam M. A. Shakir, F. H. Hussein & D. N. Taha, Iraqi Patent No. 3153, Dec. (2002).

10. J. C. Miller and J. N. Miller. "Statistics for Anal. chem.", 2nd edition, John Wily and Sons., New York.1997