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EVOLUTION AND EFFECTIVENESS OF COLORMETRIC APPROACH FOR INVESTIGATION OF CEFTRIAXONE MEDICINE AS IN PURE FORM AND IN FORMULATION VIALS

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ABSTRACT

A third-generation cephalosporin antibiotic was aceftriaxone medicine. Similar another (3rd generation cephalosporins), it has wide broad spectrum effectiveness versus gram positive as well as gram negative bacteria. Diverse approachs for analyses of the studied medicine are obtainable but are costly additionally time consumption. Therefor we have sophisticated developed novel, easy, simple as well as accurate colormetric approach for investigation of ceftriaxone medicine as pure form as well as in formulation vials by depending on a specific color-generated reaction. This reaction involves the Schiff 's base formation reaction between ceftriaxone drug with alcoholic 4- di ethyl amino benzaldehyde (DEAB) reagent to produce a new ligand that reacts with cobalt (II) ion with heating to (50°C) in acidic media to form green colored complex exhibiting λmax at 496 nm. The medicine conformed with the Beer's law with the linearity was observed between (2 –52) µg/ml additionally that correlation coefficient was 0.9992 .The analyses outcomes were supported with LOD, LOQ, accuracy, recovery studies, ruggedness as well as precision. The approach was establish to be robust as well as economical.

Keywords: Ceftriaxone, evolution and effectiveness, colormetric approach

INTRODUCTION

Antibacterial action of ceftriaxone medicine CFT is produced through the suppression of mucopeptide formation in the cell wall of bacterial as well as by joining with to the penicillin-joining proteins (PBPs) with one or more position which in benignity prohibits the end transpeptidation stride for peptidoglycan composition in the cell walls of bacterial, thus obstruction fot the biosynthesis of cell wall for bacterial. This leads to subsequent cell death by analysis due to on-going activity of cell wall autolytic enzymes continues while cell-wall assembly is arrested ceftriaxone sodium is well absorbed intramuscularly and possesses a complete bioavailability after intramuscular and intravenous

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administration. Urinary excretion is the major elimination pathway for ceftriaxone sodium. As such, 33-67% of dose is excreted in urine as unchanged drug and the remaining fraction is eliminated in faces through bile.Biliary elimination is significant for Ceftriaxone [1 and 2].

CFT is overwhelmingly utilized (in integration with macrolide as well as aminoglycoside antibiotics). The remediation of combination- obtained acquired pneumonia. It is furthermore a medicine of cucumber for the remediation of bacterial meningitis.In paediatrics, it is ordinarily utilized in pyretic toddler. It has furthermore been utilized in the remediation of leptospirosis,gonorrhea as well as lyme disease. It is likewise utilized as a habit preventative antibiotic for the patients incuring orthopedic operation [3]. Ceftriaxone (M.wt=662) chemically defined as, (IUPAC name) 6R,7R,Z)-7-(2-(2-amino thiazol-4-yl)-2-(methoxy imino) acetamido)-3-((6-hydroxy-2-methyl -5-oxo - 2,5- dihydro -1,2,4-triazine-3-ylthio)methyl)-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid 3.1/2 hydrate; with the chemical formulation $C_{18}H_{16}N_8Na_2O_7S_3$,3.1/2$H_2O$, and a calculated molecular weight of 661.59, is a third generation cephalosporin antibiotic. It has the following structural formula. Figure (1) [1,2, and 4].

Sundry analytical approaches have been introduced for the analyses of CFT, instituted based on thin layer chromatographic [5-7] derivative spectrophotometric [8], spectrophotometric [9-15], flour metric [16,17], FIA [18], high performance liquid chromatographic [19,20], ion exchange chromatographic [21], ion selective electrodes [22], polarographic and voltammetry techniques [23 and 24] and ion pair liquid chromatographic [25]. For colormetric analyses, the investigation is achieved with utilizing convenient reagent DEAB is putted for the foremost time as a derivate reagent for colormetric investigation of CFT in pure form as well as in formulation vials. The objective from the existing paper was to explain the interaction between reagent DEAB as well as CFT to produce a new ligand that complexing with cobalt chloride solution with the existence of acidic media iodate to form new color complex that utiizing for the spectrophotometric investigation of Ceftriaxone drug.

MATERIAL AND METHOD

Materials and reagents

Reagents,instrumentation as well as chemicals: devices exercised were a double-beam applied by digital enrollmentUV-Visible spectrometer (Japan), analytical balance (Sartorius BL 210S), Heating-cooling water bath (Haake, Fe_3) The chemicals which were used in the

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procedure with highly degree of purity and did not need to purification, all reagents in the research was given from (BDH reagent laboratory ,Chemicals Ltd), ceftriaxone pure drug 500 ppm solution obtained by dissolution 0.05 g of CFT in a volumetric flask 100 ml with deionized water.alcoholic (4- di ethyl amino benzaldehyde) DEAB reagent 2.0 % w/vsolution obtained by dissolution 2g of DEAB reagent in 100 ml by ethanol absolute by the volumetric flask. Hydrochloric acid 1M solution obtained by adding(4ml) form concentrated Hydrochloric acid in100ml volumetric flask ,mixed well and completed the volume by deionized water.cobalt chloride ($CoCl_2 \cdot 6H_2O$) 0.02M solution given by dissolving 0.471 g of fresh material in the 100 ml deionized water.

Assay procedure for ceftriaxone sodium in formulation vials samples

A number of vials containing CFT as ingredient active were analyzed. These are summarized in Table 1.

Dry Injection Procedure

The 0.05 g powder of every type of vials which was containing CFT as antibiotic was taken into flasks volumetric 100 ml as well as weakened up to the imprint with water solvent. At that point we computed the concentration by using the calibration graph [4].

Common procedure

Aliquots of (0.2 ml – 4.0 ml) CFT token into a collection of 25 ml volumetric flasks.To everyone, alcoholic solution for DEAB reagent 2.0 ml was used additionally 1.5 ml cobalt chloride 0.02M was added and acidic the solution by utilizing 0.25 ml for 0.5M HCl solution. The product solution was warming at 50°C for 40 minutes in the bath of water to form the color solution. the volume was brought up to tick by water as well as the colored complex absorbance was appointed at 496 nm as (λmax) contra the blank solution. The colored classes as steady for extra time high than 3 hour. The measure of CFT concetration current in the samples was checked from calibration diagram.The colour was constituted to be constant for up one 2 days [13].

RESULT AND DISCUSSION

Wavelength chosen of absorption spectra

For the measurement of the amount of drug by the studied approach, the maximum wavelength for the absorbance performs an paramount function. It is indispensable to adopt the wavelength whereover the DEAB agents as well as cobalt ion point out lower absorbance

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additionally the analyte derivative appears ultimate absorbance account in acidic media. The absorbance value of the result concentration 20 µg/ml of CFT) and (DEAB) reagent,cobalt ion derivative was registered at diverse wavelengths between 300 - 600 nm after warming to 50°C for 40 minutes using acidic media. It is obvious that the ultimate absorbance happened in visible district at 496 nm contra blank solution as well as was elected as exemplary [26]. Figure (2).

DEAB concentration influence

The influences of appending for the solutions with diverse DEAB concentration on the absorbance of 1ml of 500µg /ml CFT was checked by Table (2). The DEAB concentration was diversified by the range from (1- 6ml) of 2.0 % w/v of DEAB reagent in ethyl alcohol with a spacing of 1ml A maximum absorbance for the product solution was observed with addition of 2ml from (2% w/v) DEAB reagent solution. That was selected in the next tests [27].

Cobalt chloride concentration influence

The influences of appending for the solutions with diverse cobalt chloride concentration on the absorbance of 1ml of 500µg/ml CFT was checked by Figure (3). The cobalt chloride concentration was diversified by the range from (1-5ml) of 0.02M in deionized water with a spacing of (0.5ml). The maximum absorbance for the product solution was appeared with addendum of 1.5 ml from 0.02M of Cobalt chloride solution. That was selected in the next tests [28].

Selection of media

It was found that the presence of acidic media led to increase the intensity of absorbance for the color product more than basic and natural media, therefore some acids such as CH_3COOH, HCl, HNO_3 as well as H_2SO_4 are examined at (1M) as concentration it was found that all these acids gave the absorbance of colour product, so HCl was the best acid that gives the highest absorption which selected in the following experiments. Which was found that(0.25 ml) of this acid give high sensitivity which selected in subsequent experiments [27].

Order of mixing for the reagents influence

The influence of the order of mixing for the reagents through complexion operation has paramount function in reliabilityof results as well as augmentation of the absorbance. In the sitting project, it was appeared that the addendum 1ml of 500µg/ml CFT (D) solution

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followed by 2ml of DEAB (R) reagent solution .after that Taking 1.5mlcobalt chloride (M) added, at least the acidic solution1M HCl (A) with 0.25 ml was adding to form the acidic media, the maximum absorbance value was observed after that making the dilution. This order are used in the next tests [26]. Table (3).

Optimization of heating time and temperature:

To realize the ultimate absorbance amount for an CFT by the figuration of stationary stable color complex,the election of the best temperature as well as is fundamental.The temperature influence on the figuration of derivative was limited at 496 nm by the range from(0 - 80°C) by heating in water bath with an interval of (5°C). The heating time effect on the figuration of derivative was calculated at 496 nm from(0 - 80min) also with a spacing of (5 min). A maximum absorbance was appeared after warming for (40 minute) at (50°C) additionally was believed as ideal. that was used in the following tests [28]. Table (4).

Solvents infuence

The different solvents influence like ethanol, 2-propanol, methanol, 1-butanol, acetone, chloroform, dimethyl sulphoxide, benzene, teri butyl alcohol, nitrobenzene, formic acid, dimethyl formamide, di ethyl ether and carbon tetrachloride on the absorbance was studied. Table (4) shows the effect solvent, water was the best solvent, which giving very high intensity of maximum absorbance water is achieved to be a decent solvent from the point perspective of economy as well as sensitivity. Water utilized for the dilution to the mark of (25 ml conical flask). After heating the result solution for (40 min) at (50°C) [26].

Beer's law calibration diagram

The variation in the CFT concentration influence on the color produc absorbance was checked. After applying the perfect circumstances was showing by the procedure, a linear calibration diagram was acquired which conformed with the Beer's law through the range of concentration (2 – 52 µg.ml^{-1}) of CFT antibiotic with coefficient of investegation r^2 (0.9992) figure (4).

The Sandell's sensitivity was observed(4.8×10^{-5}g.cm^{-2}). The highly molar absorptivity for the color product was founding 2.1184× 10^4 L. mol^{-1}.cm^{-1}. (LOD) limit of detection as well as (LOQ) limit of quantitation were checked by utilizing the equation LOD = 3 × s/S additionally the equation LOQ = 10 × s/S, where s is standard deviation for the intercept, the strength line slope was S.

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Effectiveness

Accuracy

To appreciate the accuracy for of the suggestted approach, recovery considerations were preformed by three various scales i.e. (4, 20 and 40) µg.ml^{-1}.To the pre-tested sample solution a recognized quantity standard medicine solution was adding added at three various scales,the absorbance was registered.The % recovery was then recorded by the equation % Recovery = [(A – B) / C] x 100. Where A is overall total amount of medicine assess ; B is the medicine amount establish on pre tested footing; C is the pure medicine amount added to formulation vials [27].

Precision

The approach precision is checked as during the day as well as between the days.These precisions was calculated by analyzing the identical concentration for the solutions every day for 3 days. In medium precision project, % R.S.D. measurements were not highly than 2.0 % in every one the statuses [28]. Table (6).

Stoichiometric proportion investegation

Utilizing the ideal situations, the new ligand absorbance was measuring the various concentration medicine with DEAB were installed to alter with the stoichiometric of proportion the CFT medicine as well as DEAB by utilizing mole ratio method and Job's method. The 1:1 was the mole ratio that lead to obtain the strongly absorbance measure for the new ligand, additionally for this reason that was chosen as the stoichiometric proportion for the next investegations. The stoichiometric proportion between new ligand CFT : DEAB and Co (II) of investigated macrolides in water solvent were employed to determine by mole-ratio and Job's method of continuous variation as follows. Confirms that the ratio of product complex new ligand CFT : DEAB : Co(II) is equal to 1:1. The products formed was water soluble. A reactions mechanism based on the above reactions is shown in schemes figure(5) [26]. The stability constant for the product compounds was figured by looking and measuring the absorbance of a solutions which including stoichiometric measure of medicine with DEAB with the perfect quantity 1ml of 2×10^{-3}M from medicines, and DEAB solution with other solution for cobalt ion containing a five-fold excess from the starting concentration. The average for calculated stability constant for the colour results in water under the characterized experimental circumstances was 2.41×10^{6} l^{2}.mol^{-1}. The interaction may happened as obtained by the next Schemes [27-29] figure (6).

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Interference study

Under the optimized experimental conditions, the effects of additives associated with CF T medicine in its formulations were investigated using the developed method. The results obtained are indicated in Table. 7) and the resultsare indicating that the method is not suffering anyinterference from common exci pients and other substances added to vials preparations. the method was applied by.1ml of 500ppm CFT and 1ml of each excipients with concentration 5000 ppm was connected for the study of interferences after that dilution to the sign of volumetric flask 25ml.A grade of impedance was believed to make the procedure more satisfactory if the mistake was not more than ± 2% in respect to the normal No obstructions were seen on the investigation of drugs within a sight of the studied excipients [30]. (Three determinations Average).

Application

The was exercised to assess of CFT in different formulation vials(Table 8) ,a present approach was applying for the assay of five samples and pure form for CFT medicine (vials) and comparison with the British pharmacopoeia method (Table. 8). The results shown give a reproducible and accurate result. The validation of the present suggestted approach was confirmed by applying official approach that were gotten from (2009) British [4].

The outcomes were as well matched statistically with variance ratio F-test additionally student t-test with those acquired by British pharmacopeia at 95% confidence scale with five degrees for freedom, as witnessed in (Table. 9). The result appeared that the F-test as well as t- test were minimal than the impractical account *(F*=5.05 , *t*=2. 29). The values were for proposed method *(F*=1.66 , *t*=0.89) as well as the values for of the official method were *(F*=1.66, *t*=1.09) marking that there was no considerable variance between the suggestted approach as well as official approach.

CONCLUSION

The suggestted approach was emphasized with a thematic of improving sentient, unpretentious as well as authoritative analytical approach hassling UV-Visible colometric approach for investegation of ceftriaxone as pure material as well as formulation vials. The approach has appropriatly perfect precision, accuracy as well as allowed as a less cost efficient effective than another approaches. The advanced approach may averted the infuence of interferences from connected substances which may soak up in the UV district. The

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analytical approach is sentient, unpretentious, specific as well as fast. Moreover it can be suitable utilized for the monotone analyses additionally the quality monitoring of ceftriaxone in formulation vials.

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Table 1. Formulation vials studied for Ceftriaxone sodium

formulationials samples	Declared composition	Company
Ceftriaxone sodium (Philtriaxone)	Per vial 1g ceftriaxone sodium	Binh duong company, Vietnam
Ceftriaxone sodium	Per vial 1g ceftriaxone sodium	Sanaita,Pharmaceutical GMBH company (Werne Germany)
Ceftriaxone sodium (Roth)	Per vial 1g ceftriaxone sodium	Pharma Roth Gmbh company (Germany)
Ceftriaxone sodium (Enoxirt)	Per vial 0.5g ceftriaxone sodium	Gulf Pharmaceutical industries (UAE)
Ceftriaxone sodium (Rameceftrix)	Per vial 1g ceftriaxone sodium	Tenth of Ramadan for pharm.industries company (Egypt)

Table 2. Influence of volume of DEAB reagent adding ml in color complex formed

(DEAB) reagent solution adding (ml) Volume of	Absorbance
1	0.665
2	0.701
3	0.621
4	0.587
5	0.532
6	0.463

Table 3. Order of mixing the reagents influence

No.	mixing order	Abs.
1	D+R+M+A	0.720
2	R+M+D+A	0.615
3	M+D+R+A	0,510
4	A+D+M+R	0.471
5	D+M+R+A	0.533

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Table 4. Optimization of heating time and temperature on absorbance of color product

T(min)	Abs(25°C)	Abs(40°C)	Abs(50°C)	Abs(60°C)	Abs(70°C)	Abs(80°C)
5	0.083	0.211	0.241	0.233	0.159	0.112
10	0.121	0.258	0.298	0.331	0.236	0.166
15	0.156	0.299	0.351	0.387	0.289	0.213
20	0.185	0.323	0.398	0.453	0.333	0.254
25	0.209	0.474	0.465	0.501	0.377	0.271
30	0.231	0.512	0.562	0.579	0.411	0.313
35	0.288	0.598	0.632	0.607	0.421	0.334
40	0.331	0.625	0.711	0.641	0.443	0.351
45	0.331	0.627	0.710	0.639	0.441	0.350
50	0.331	0.626	0.709	0.640	0.443	0.344
55	0.330	0.626	0.711	0.638	0.439	0.326
60	0.332	0.625	0.710	0.640	0.433	0.316
65	0.331	0.627	0.711	0.636	0.423	0.305
70	0.330	0.626	0.710	0.634	0.401	0.288
75	0.331	0.628	0.709	0.634	0.389	0.267
80	0.331	0.627	0.711	0.635	0.378	0.251

Table 5. Shows the effect solvent on (λ_{max}) and absorbance for the color complex

Solvent	**λ_{max} ,nm**	**Absorbance**
Ethanol	468	0.571
Methanol	432	0.421
2- propanol	398	0.221
1-butanol	366	0.310
Acetone	400	0.128
Chloroform	350	0.088
Dimethyl sulphoxide	362	0.131
Dimethyl formamide	380	0.211
CCl_4	330	0.013
Benzene	346	0.140
nitrobenzene	336	0.102
Teri butyl alcohol	390	0.344
Formic acid	352	0.186
Di ethyl ether	344	0.211
Water	496	0.711

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Table 6. Optical analytical characteristics and Precision for the studied method

Optical analytical characteristics	Values
λmax (nm)	496
Molar absorptivity (lit. $mol^{-1}.cm^{-1}$)	2.1184×10^{4}
Beer's Law limits (µg/ml) (x)	2 – 52
Sandell's sensitivity $\mu g/cm^{2}$	$4.8\times10^{-5} g.cm^{-2}$
(LOD) Limit of detection ($\mu g.ml^{-1}$)	0.421
(LOQ) Limit of quantification ($\mu g.ml^{-1}$)	1.121
Regression equation (y = bx + a)*	y = 0.032 x + 0.0484
Slop (b)	0.032
Intercept (a)	0.0484
Correlation coefficients (r)	0.9992
RSD%	0.921
Recovery %	99.970
Confidence limits with 0.05 level	±0.0018
Confidence limits with 0.01 level	±0.0027

Table 7. Investegation of 20ppm CFT in the presence of excipients

Interference	**% Error**	**% Recovery**
Lactose	- 1.160	98.840
Talc	- 1.080	98.920
Starch	+ 1.120	101.120
Acacia	+ 1.230	101.230
Sucrose	- 1.500	98.500
Glucose	- 1.150	98.850
magnesium stearate	+ 1.400	101.400
Tween 80	- 1.010	98.990
benzoic acid	- 1.150	98.850
Aspartate	- 1.230	98.770
microcrystalline cellulose	-0.100	99.900
PVP	+ 1.070	101.070

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Table 8. The present method was applying for the assay of five samples and pure form for(CFT) drug

Procedure Applied	Pharmaceutical Formulation of (CFT)	Recovery%	%E Relative Standard error	Relative Standard Deviation %
Proposed method	PureURE Ceftriaxone sodium	99.370	- 0.630	0.921
	Ceftriaxone sodium (Philtriaxone)	99.100	- 0.900	0.882
	Ceftriaxone sodium	98.440	-1.560	0.971
	Ceftriaxone sodium (Roth)	100.830	+0.830	0.7717
	Ceftriaxone sodium (Enoxirt)	98.510	- 1.490	0.832
	Ceftriaxone sodium (Rameceftrix)	101.090	+1.090	0.921

Table 9. the official method was applying for the assay of five samples and pure form for(CFT) drug

Procedure Applied	Pharmaceutical Formulation of (CFT)	Recovery%	%E Relative Standard error	Relative Standard Deviation %
British Pharmacopoeia method [4]	PureURE Ceftriaxone sodium	99.130	- 0.870	1.122
	Ceftriaxone sodium (Philtriaxone)	101.230	1.230	0.911
	Ceftriaxone sodium	98.140	- 1.860	0.982
	Ceftriaxone sodium (Roth)	98.200	- 1.800	1.077
	Ceftriaxone sodium (Enoxirt)	98.670	- 1.330	1.032
	Ceftriaxone sodium (Rameceftrix)	98.890	- 1.110	0.944

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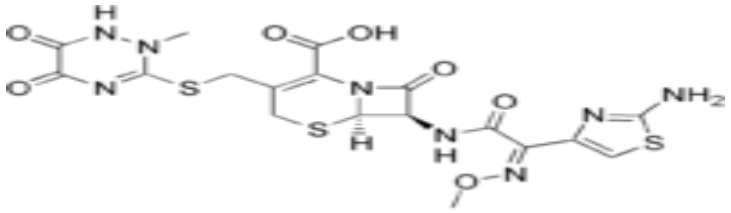

Figure 1. The chemical structures of Ceftriaxone drug

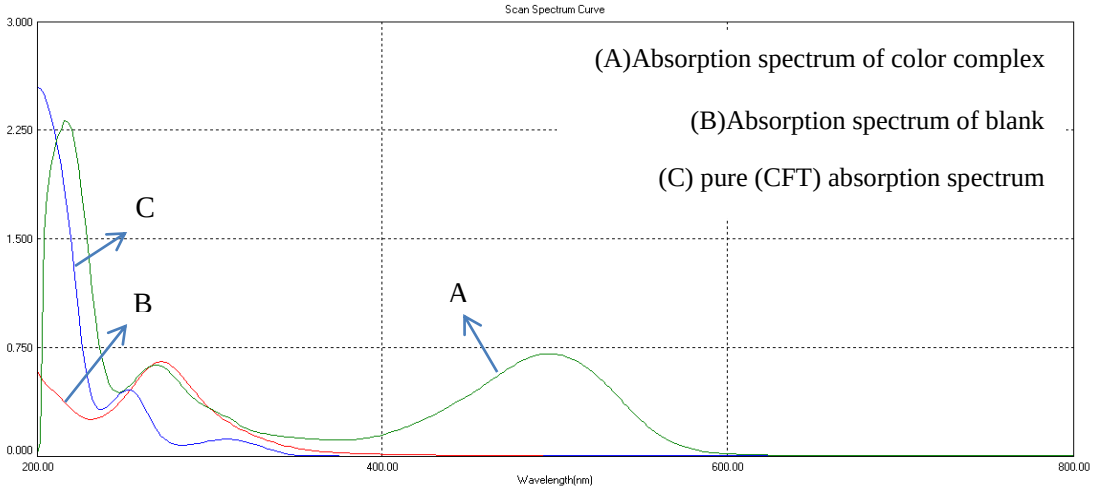

Figure 2. (A) absorption spectrum of color complex formed by(CFT) (20mg/ml), (DEAB) and cobalt ion solution in acidic media, (B) absorption spectrum of (DEAB) and cobalt ion solution in acidic media, (C) pure (CFT) absorption spectrum

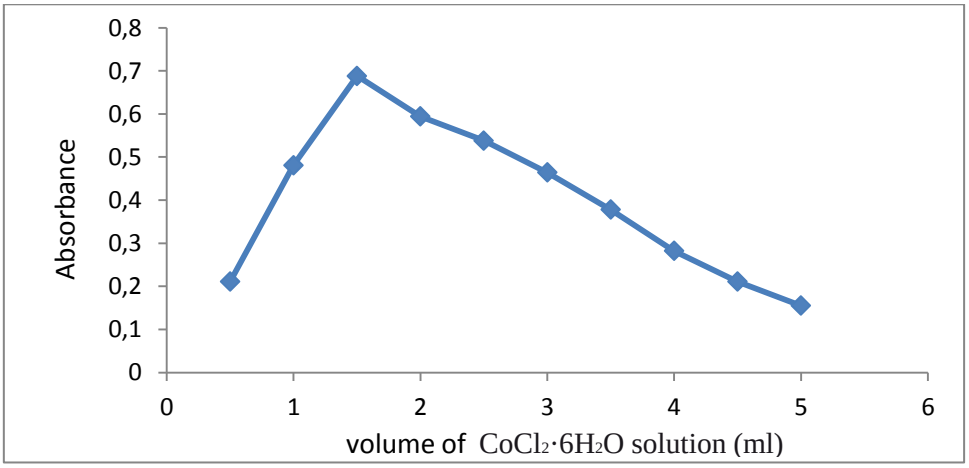

Figure 3. Cobalt chloride concentration influence

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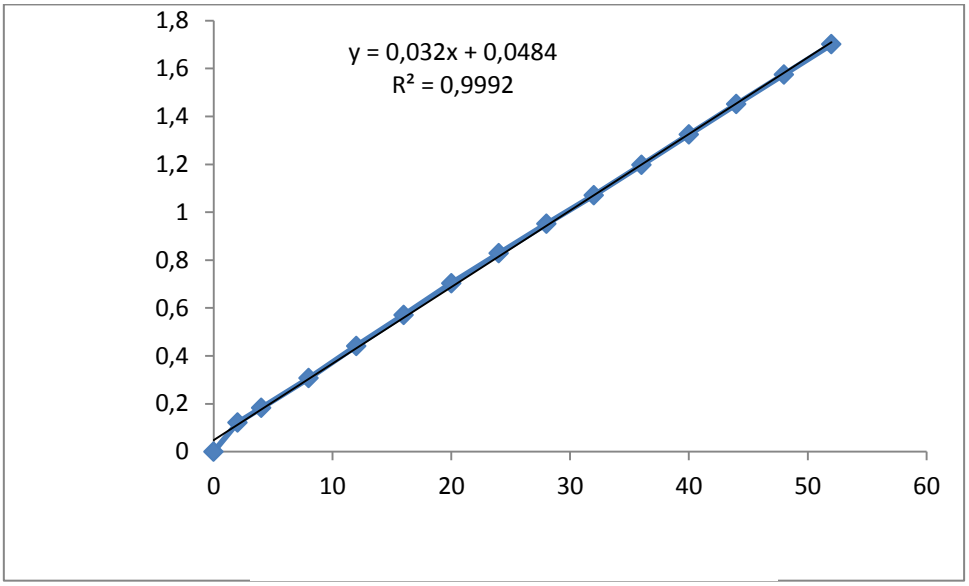

Figure 4. The calibration graph of (CFT)