ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Rapid Determination of Bi (III) and Al (III) in Pharmaceutical Formulations using Organic Reagent Spectrophotometrically

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Abstract

A new trend describes the development and validation of a simple, sensitive and selective spectrophotometric methods for the determination of Bi (III) and Al (III) in pharmaceutical formulations have been conducted. In this paper, 4-(4 acetamidophenylazo) pyrogallol was synthesized as a new organic compound, 4-APAP, by coupling pyrogallol with diazotized p- aminoacetanilide in medium of controlled pH. 4- APAP was characterized by techniques of FT-IR, H-NMR, GC-Mass, TG and DSC thermal analysis methods. Solvatochromic behavior in solvents of various polarities was also investigated.

Keywords: *Pharmaceutical applications, Pyrogallol, Bi (III), Al (III).*

Introduction

For many years, organic reagents play an important role in the chemical and physicochemical methods of analysis. They are employed for qualitative or quantitative determination of chemical elements and compounds (both organic and inorganic), as well as for separation, concentration, masking and other auxiliary operations that precede or accompany the analysis. Theoretically, the possibilities of design of new organic compounds for analytic purposes are boundless; however, in practice only several dozens of classes of chemical compounds are effective as organic reagents [1-3].

The requirements for the organic reagent are dictated by the analytical method used. The reagent for the determination of metals should, first of all, contain a proper functional analytical group that enables its interaction with the determined element and subsequent observation of the respective analytical signal. Currently, several tens of such functional analytical groups are known and documented [4-7]. Aluminum is a versatile metal, which has tremendous usage in the realm of industries and other spheres of life. Our earth is a storehouse of this metal in abundance and it is used in every walk of life like engineering, making artificial gems and many other things. Today, this cost-effective metal is widely available throughout the world and its alloys are used extensively for adding strength and utility [8]. Aluminum also used for dialysis dementia [9]. High amount of aluminum is toxic for human [10]. On the other hand, micronutrient roles of the metal ion are also well recognized. Aluminum also found in over the counter medicinal, such as antacids and buffered aspirin, is used as a food additive, and is found in a number of topically applied consumer products such as antiperspirants, and first aid antibiotic and antiseptics [11].

Bismuth is environmentally important [12], and is of biological interest due to their possible binding to thiolate ligands to form metal-thiolate clusters [13]. The most commonly used methods for determination of various metal ions are electroanalytical procedures [14], atomic absorption spectrometry (AAS) [15], atomic emission spectrometry (AES) [16] and mass spectrometry (MS) [17]. However, these techniques have some disadvantages, such as complicated operation, high cost of maintenance, expensive apparatus and requiring well-controlled experiment conditions. Spectrophotometry is an essential trace analysis technique and is one of the

most powerful tools in chemical analysis. Earlier several published spectrophotometric methods based on use of various reagents are reported for the determination and detection of Al [18-42] and bismuth [43-51].In the present study; we used the organic reagent for the determination and spectrophotometric analysis of Bi (III) and Al (III) in their pharmaceutical preparations.

Experimental

Apparatus

UV-visible spectra were measured with matched 1 cm quartz cells by T80 UV-visible spectrophotometer, PG instruments Ltd., UK. PH was monitored using 340i pH-meter WTW, Germany. Oven BS Size Two, Germany was also used.

Reagents

All chemicals were of analytical-reagent grade and supplied from the stated companies. HCl supplied from G.C.C, 4-Aminoacetanilide from C.D.H, Pyrogallol from Fluka, NaOH, THF from B.D.H, $NaNO_2$ from Merck, Double distilled water was used throughout.

Synthesis of 4 (4-acetamidophenylazo) pyrogallol

Diazotization Reaction

Solution (1) was prepared by dissolving 1.4 g (0.02 mol) of sodium nitrate in 10 ml D.W.

Solution (2) was prepared by dissolving 3g (0.02 mol) of P-amino acetanilide in mixture of 5 ml conc. HCl and 20 ml of acetic acid and 25 ml D. W.

The diazotization reaction was accomplished by addition solution (1) to solution (2) at temperature not exceed 5°C for 30 min.

Coupling Reaction

Alkaline solution of pyrogallol: 2.5g (0.02mol) of pyrogallol was dissolved in 50 mL D.W.

The reaction of coupling was achieved by reacting the diazonium salt which was prepared above directly with alkaline solution (not exceed pH=6.0) of pyrogallol at 0°C. The product was left over night, then filtered and purified with absolute ethanol (compound 2 in Fig. 1).

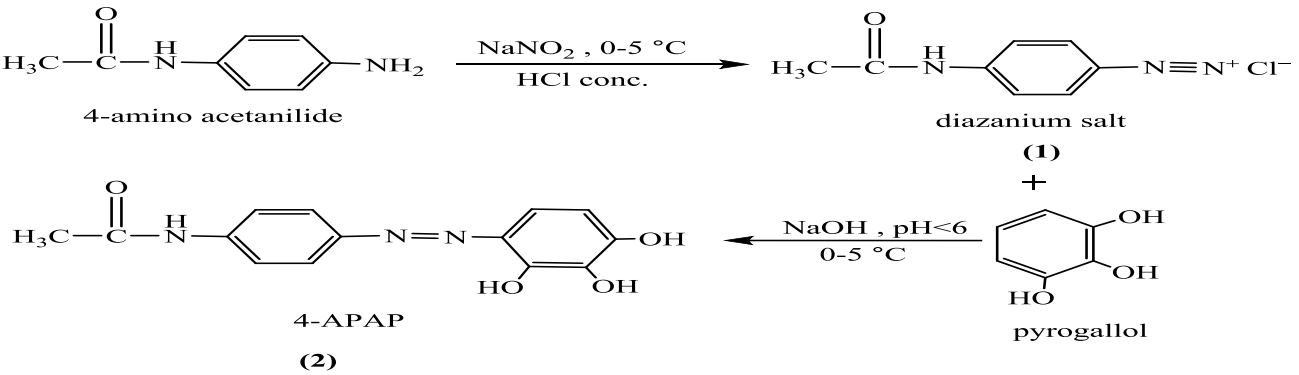
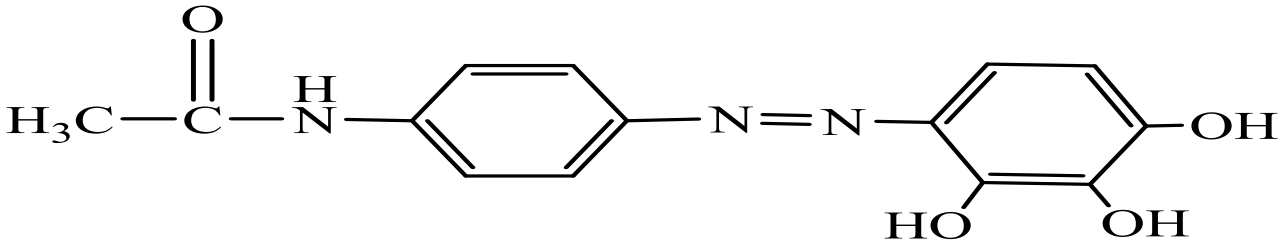


Fig.1: Synthetic rout of 4-APAP

Results and Dissection

Physical and Chemical Properties of 4-APAP

The reagent is Dark-brown powder which was slightly soluble in water. It is soluble in methanol, ethanol, acetone, cyclohexanol, dioxane, Di methylsulphoxide (DMSO), 2-propanol, n-propanol, ethylacetate, acetonitrile, butanol, acetic acid, tetrahydrofuran (THF), and slightly soluble in ethylenechloride, toluene, chloroform, benzene. It is purple in alkaline solutions, red-orange in neutral solutions and yellow in acidic solutions.



The chemical structure and the purity of the obtained compound were confirmed by its melting point, IR, UV/Vis, H-NMR and Carbon 13 and TG-DSC analysis. 4-APAP melts at 196-198 °C.

UV- Visible Spectrum of 4-APAP

The orange solution for 4-APAP dissolved in ethanol showed peak at 391 nm assigned to absorption of N=N group, Fig 2, moreover, such absorption emphasize that compound was formed since this band is not found in all reacting molecules.

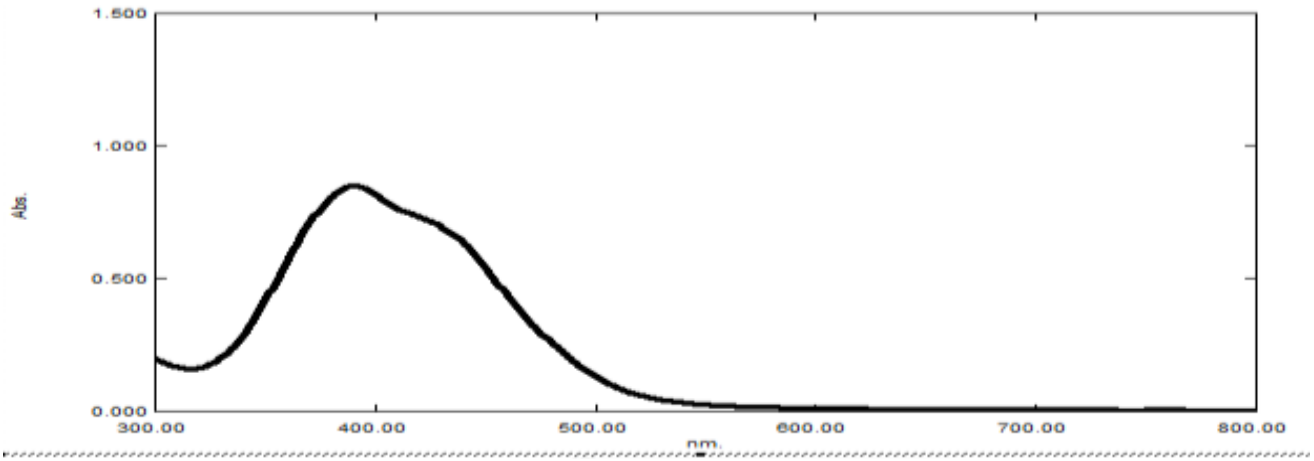


Fig.2: Absorption spectrum of 4-APAP in ethanol

The dissociation constant of 4-APAP were evaluated by a spectrophotometric method from the individual formation regions of the absorbance -pH curves by graphical analysis. The values of pKa1, pKa2, were found to be 1.2 and 6.5 respectively.

Preliminary Tests of Reagent with Ions

The primary investigations for 4-APAP with element ions under definite conditions for reaction such as concentration, temperature and pH. The following ions studied with 4-APAP;

Sn^{2+}, Al^{3+},Ag^{+},Mg^{2+}, K^{+}, Ni^{2+},Mn^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Cu^{+}, Zn^{2+}, Hg^{2+}, La^{3+}, Sr^{2+}, Pd^{2+}, Cr^{6+}, Ca^{2+}, Cd^{2+}, Pb^{2+}, Fe^{2+}, Bi^{3+}, NH_4^{+}, Na^{+}, K^{+},Ti^{3+}, Li^{+}.

These ions which showed no reaction with 4-APAP, depending on take place of change of color after mixing reactants, only Al^{3+} and Bi^{3+} were reacted with 4-APAP. Therefore the synthesized compound acts as specific reagent for these two metal ions.

Spectrophotometric Studies with Bi (III) and Al (III)

A maroon colored product formed when 4-APAP solution is mixed with solution of Bi(III) and Al(III) without any modification of pH. The absorption maximum for the colored product is found to be 506 and 497nm, Figures (3) and (4) respectively. The absorption maxima of aqueous solutions of pyrogallol and 4-aminoacetanilide are differing largely from that of their corresponding complexes due to the formation of coupling product. This reaction is exploited to develop a spectrophotometric method for the determination of Bi (III) and Al (III).

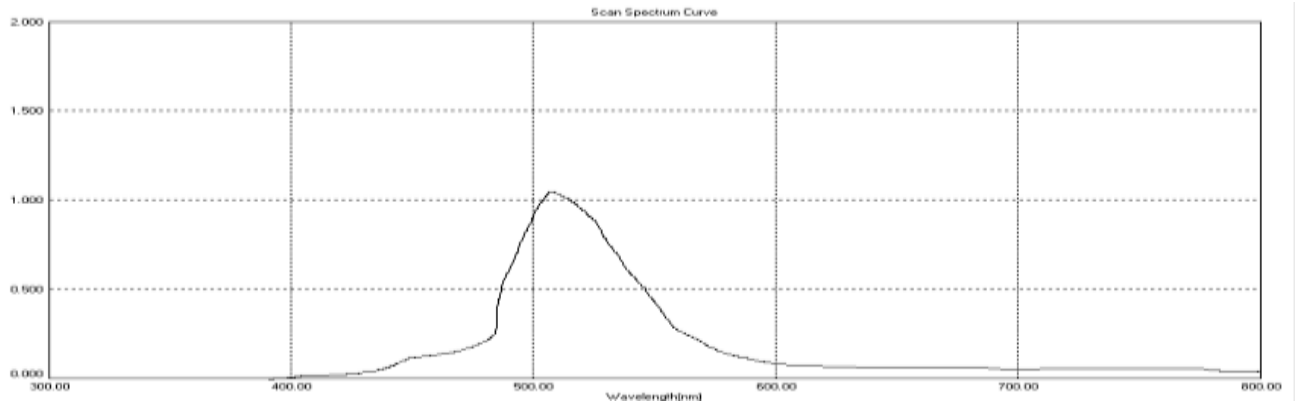


Fig.3: Absorption spectrum of bismuth complex

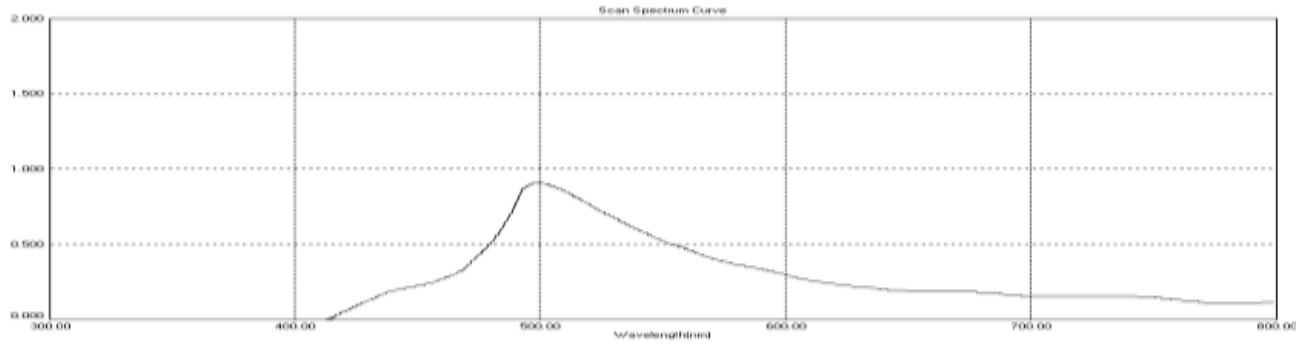


Fig.4: Absorption spectrum of aluminum complex

Optimization of the Experimental Conditions

Effect of Volume Reagent

Table (1) shows change of the absorption values of the Bi(III) and Al(III)solution with the addition of different volumes of the reagent at (3×10^{-4}) M. The table shows that the best reagent volume to complete the reaction is (2.5) mL when it is added to (1) mL of the bismuth ion solution at (100) µg.mL^{-1} at the maximum absorption wavelength, 506nm and (3.0) mL of regent solution with aluminum ion at 497nm.

Table 1: The best volume of the reagent require to complete the reaction of Bi (III) and Al (III) with 4-APAP

Volume of reagent,3×10^{4}M	Absorption value to 1mL of 100µg.mL^{-1} Bi^{3+} at 506 nm	Absorption value to 1mL of 100µg.mL^{-1} Al^{3+} at 497 nm
0.5	0.012	0.160
1	0.342	0.252
1.5	0.774	0.663
2	1.162	0.990
2.5	1.504	1.357
3	1.422	1.398
3.5	1.515	1.356
4	1.512	1.360

Effect of Temperature

The effect of temperature on complex formation was studied at different temperatures. Figures (5) and (6) show effect of temperature on bismuth and aluminum complexes respectively. The complex remained stable at highest values of absorbencies between (20-40) ºC; below this range the stability decreased due either to incomplete formation or decomposition of the product. In subsequent experiments, 25 ºC fixed as optimum value for both complexes.

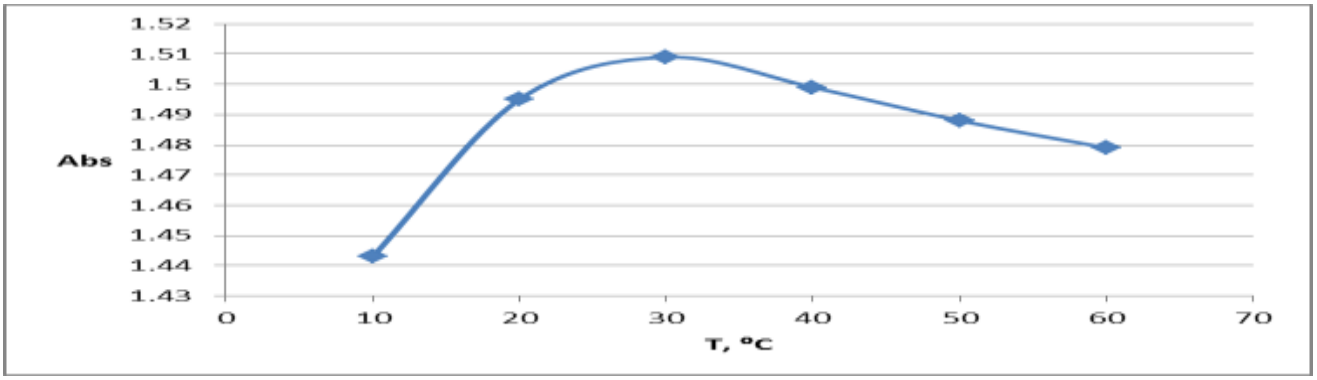


Fig.5: Effect of temperature on Bismuth complex

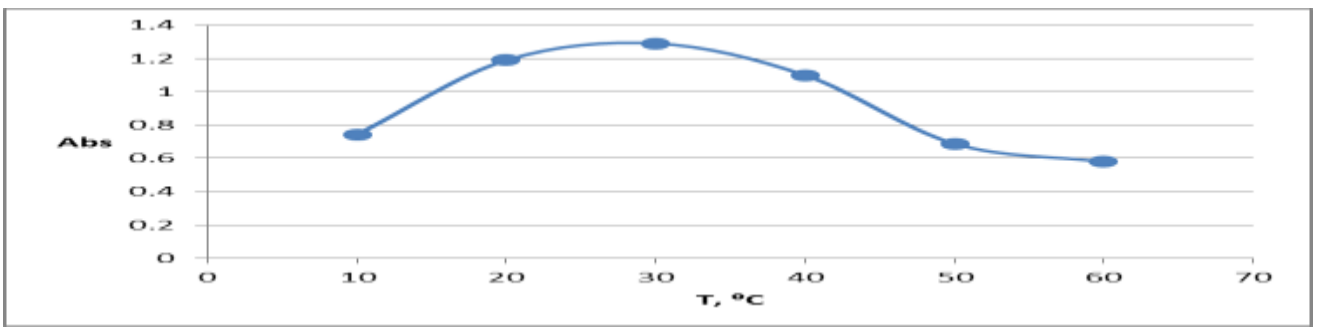


Fig.6: Effect of temperature on Aluminum complex

Effect of Time: Stability of Complex

Figures (7) and (8) show the absorption change of bismuth and aluminum complexes by respectively with increasing the time period. It is noted that the absorption is almost constant to a period of time up to 24 hours, which indicates that the complexes are highly stable. The color of the formed complex reached highest intensity with maximum absorbance at 5 minutes, therefore this time was selected as optimum value to complete reaction between bismuth ion and aluminum ion with the reagent.

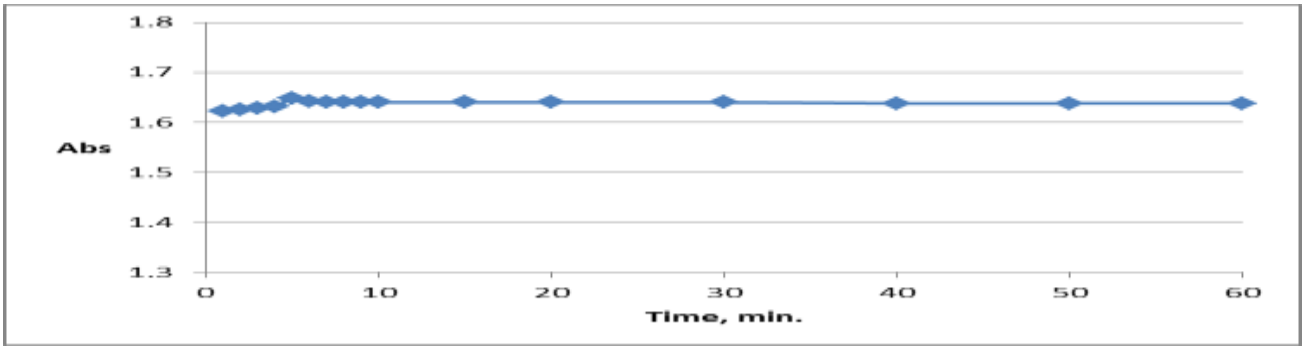


Fig. 7: Effect of time on Bismuth complex

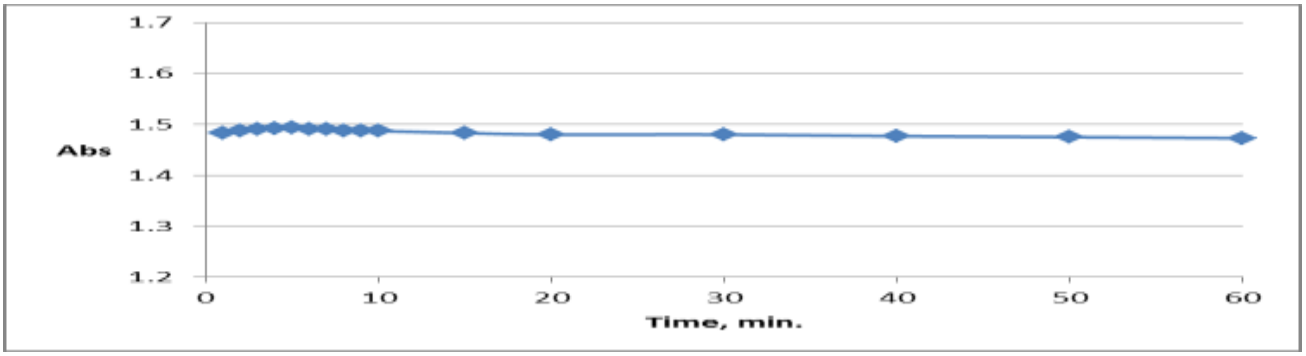


Fig. 8: Effect of time on Aluminum complex

Effect of PH

Figures (9) and (10) show the effect of pH on bismuth and aluminum complexes respectively. The best value for complex is around neutral value. Therefore pH of 6.0 was maintained for proceeding further experiments this value was considered as optimum.

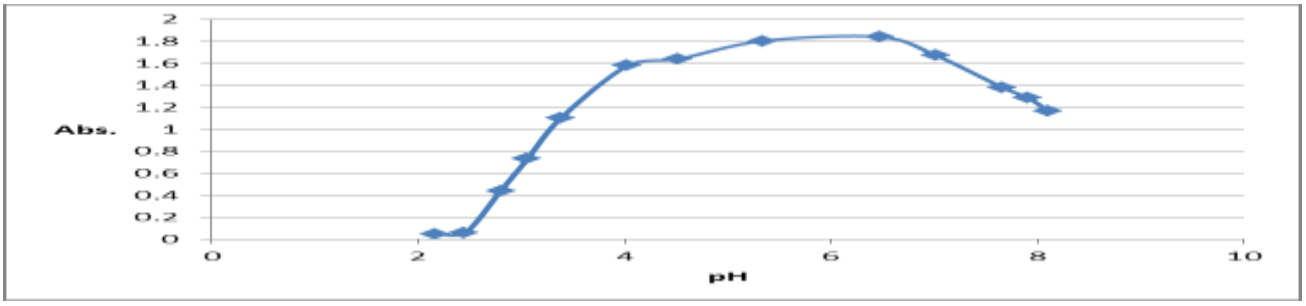


Fig. 9: Effect of pH on bismuth complex

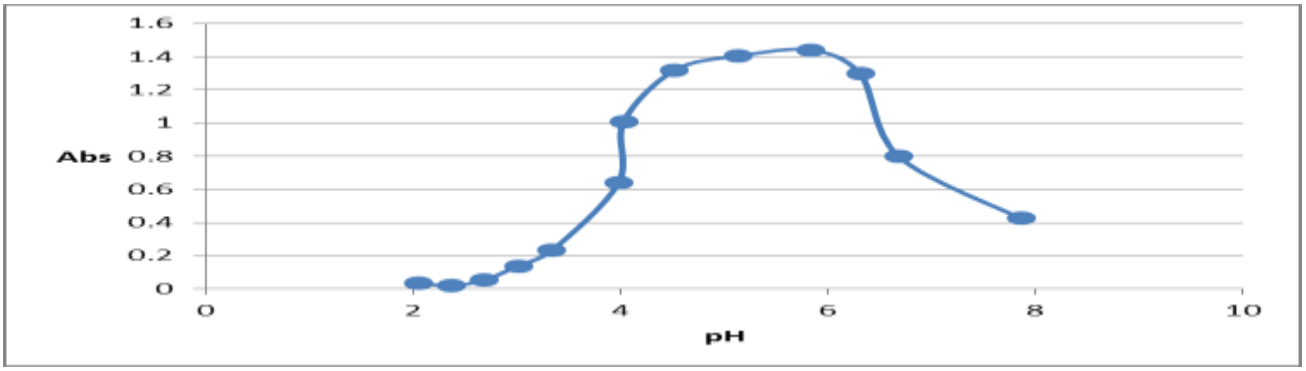


Fig. 10: Effect of pH on aluminum complex

Calibration Curve

Under the optimum conditions which studied above, standard calibration curve has been constructed for the colored product (complex). Figure (11) shows calibration curve for Bi (III) which obey to Beer's law within range of concentration (0.3-13)µg.mL^{-1} at 506 nm with correlation coefficient 0.9936. Moreover, other analytical parameters are calculated and tabulated in table(1).The results shown in table (1) make this analytical method with good performance for Bi(III) determination at low concentrations. Further, the proposed method for Bi (III) determination is highly precise and satisfactorily accurate.

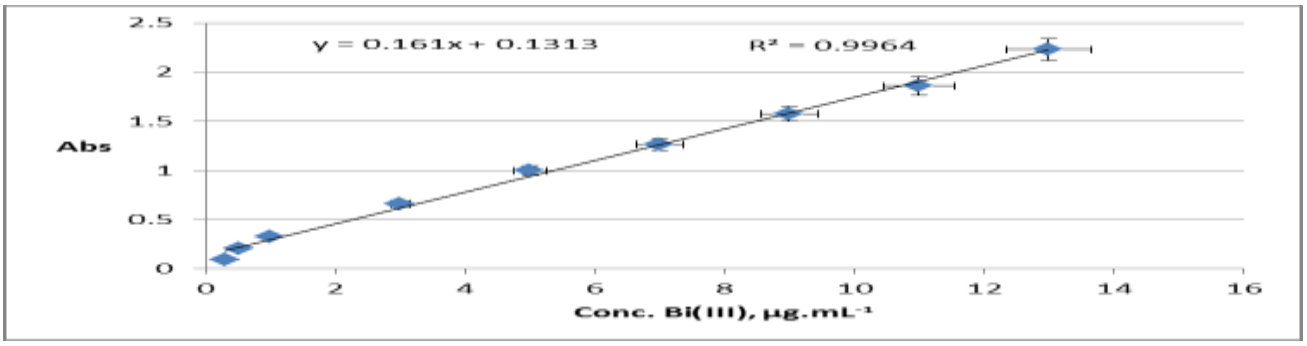


Fig 11: Calibration curve for bismuth (III)

For Al (III), standard calibration curve has been constructed by reacting with4-APAP. Figure (12) shows calibration curve for Al (III) which obey to Beer's law within range of concentration (0.5-11) µg.mL^{-1} at 497 nm with correlation coefficient 0.9995. Other analytical parameters are calculated and tabulated in Table(1).The results shown in this table make this analytical method with good performance for Al(III) determination at low concentrations.

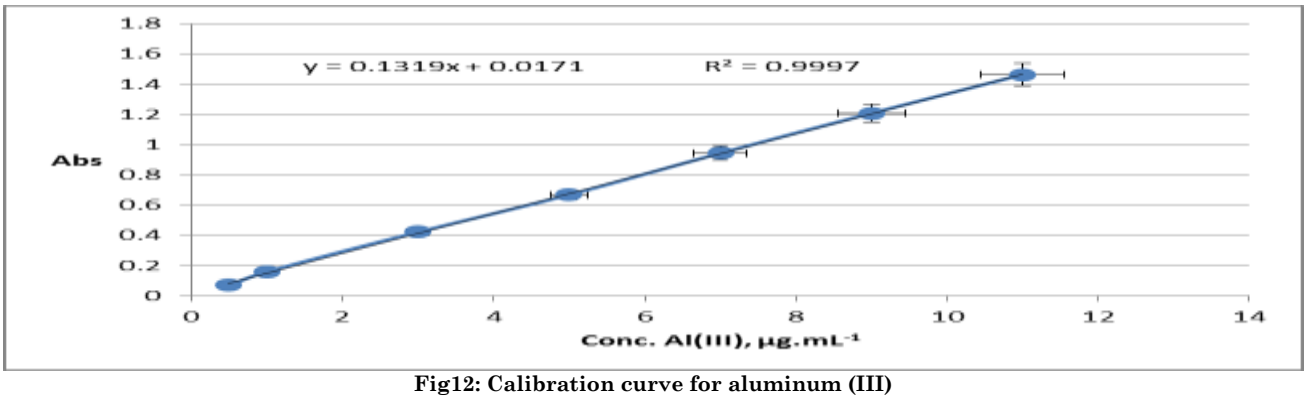


Fig12: Calibration curve for aluminum (III)

Table 1: Analytical performance for Bi (III) determination

Parameter	Value	Value
Beer's law limit (µg.mL^{-1})	0.3 – 13	0.5 – 11
Molar absorptivity(L.mol^{-1}.cm^{-1})	3.365×10^4	0.356×10^4
Sandell's sensitivity(µg.cm^{-2})	0.006	0.008
Detection limit (µg.mL^{-1})	0.013	0.018
LOQ (µg.mL^{-1})	0.043	0.059
Correlation coefficient (r)	0.9982	0.9998
Determination coefficient(r^2)	0.9964	0.9997
Regression Equation*	Y = a + bX	Y = a + bX
Slope(b)	0.161	0.1391
Intercept(a)	0.1313	0.0171
RSD%, 6.0µg.mL^{-1}(n=10)	1.46 %	1.72
E_{rel}%**	2.57, -1.89, -2.67	-2.17, 3.62, 1.09
Rec%	102.57, 98.11, 97.33	97.83, 103.62, 101.09

* Y is the absorbance and X the concentration in µgmL^{-1}.
** E_{rel}% and recovery% were calculated for conc. [2.3, 5.7, 9.4]µg.mL^{-1} of Bi(III) and E_{rel}% and recovery% were calculated for conc. [2.0, 4.5, 8.0]µg.mL^{-1} of Al(III)

Estimation the Composition of the Complex

Figures (13) and (14) show the methods of continuous variations and the molar ratios, respectively, of the bismuth complex with the reagent while Figures (15) and (16) belong to aluminum complex. The results refer to that the ratio of both Bi (III):4-APAP and Al (III):4-APAP is1: 2.

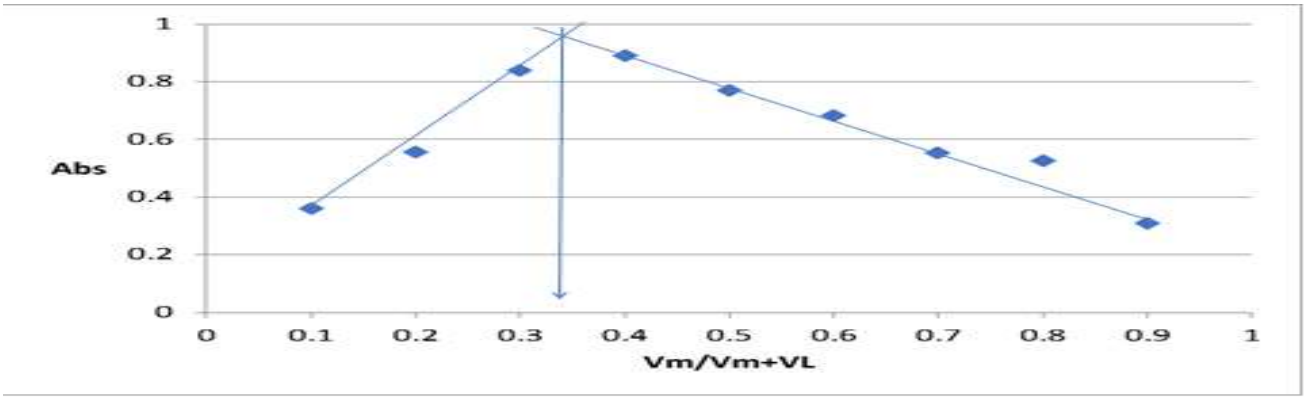


Fig 13: Method of continuous variations of Bi^{3+} complex at pH = 6

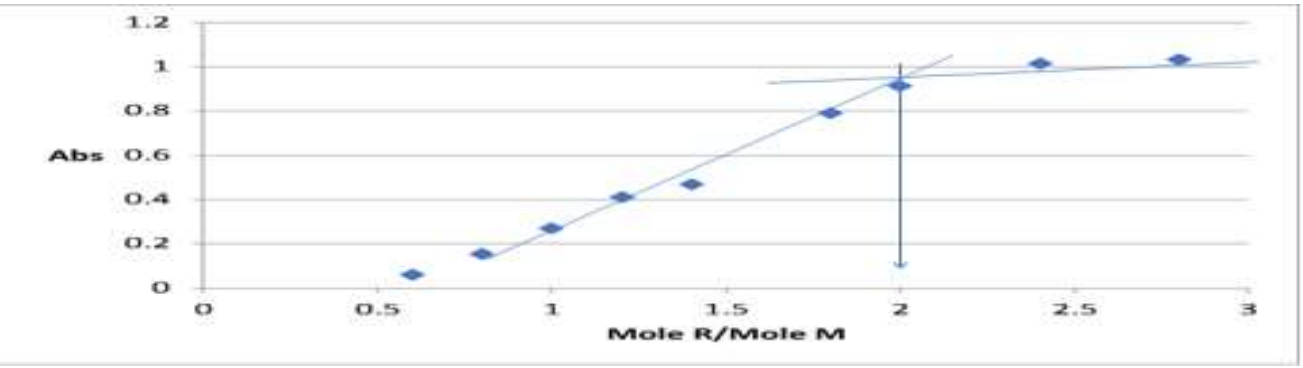


Fig14: Molar ratio method for Bi^{3+} complex at pH =6.0

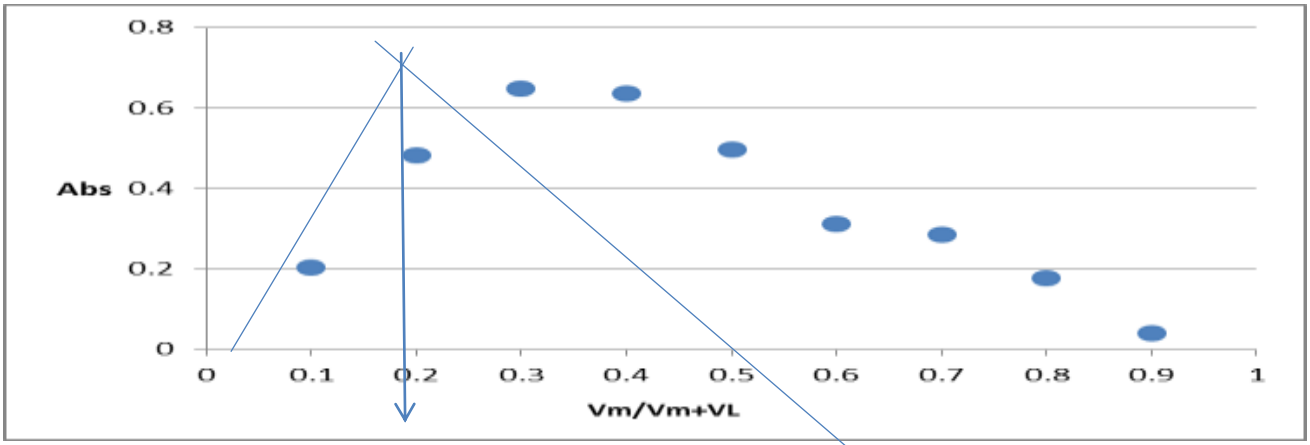


Fig 15: Method of continuous changes of $Bi^{3+}$and Al^{3+} complexes at pH= 6.0

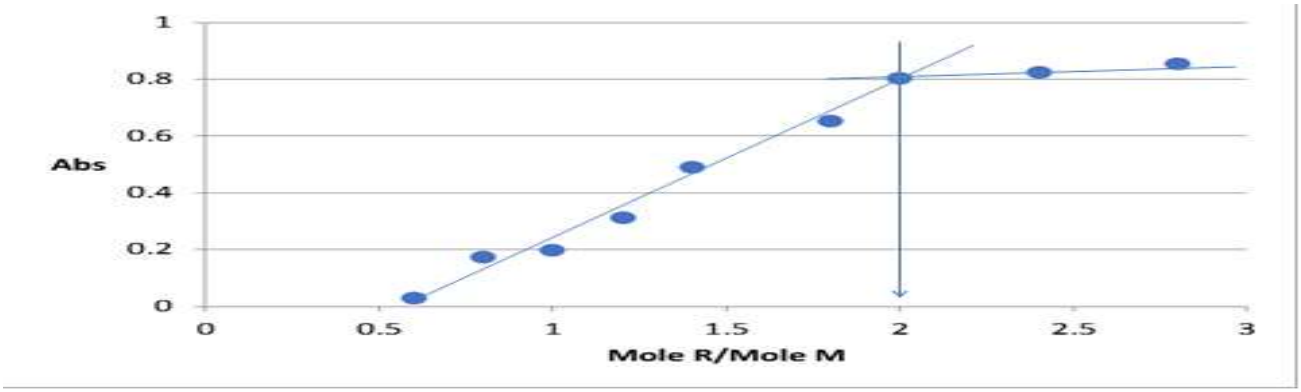


Fig 16: Molar ratios method for Bi^{3+} and Al^{3+} complexes at pH =6.0

Calculation Degree of Dissociation and Estimation of Stability Constant of the Complex

The degrees of dissociation of the bismuth and aluminum complexes were calculated according to equation (1). It was found that the degrees of dissociation are (**α**= 0.871) and (α=0.875) for two complexes respectively. Value of the stability constant($k_{st.}$), which conducted from equations (2) and (3), is 5.4×10^{6} liter.mol^{-1} bismuth complex and $k_{st.}=5.2\times10^{5}$ liter. Mol-1 for aluminum complex, that's indicates that the complexes are highly stable and can be studied easily.

$$\alpha = = \frac{Em - Es}{Em} \qquad \text{.....(1)}$$

$$K_{ins.} = \frac{(ac).(nac)^{n}}{c(1-\alpha)} \qquad \text{..... (2)}$$

$$K_{st.} = \frac{1}{Kins} \qquad \text{..... (3)}$$

Application of the Proposed Methods

To examine the validity of the present method, it has been applied to the determination of bismuth ion and aluminum ion in pharmaceutical forms (two marks of ointment, PROCTO-CINOLONE and Proctoyat, for Bi (III) and Aciloxplus Tablets-Antespin Tablet- Sucralfate (solution) for Al (III). The reliability of the method to analyze these real samples are checked by recovery experiment, Table 2 and Table 3. The recovery is close to 100% and indicates by applying the proposed procedure, good recovery is obtained.

Table 2: Determination of Bi^{3+} in dosage forms using the proposed method

Sample	Present, µg.mL^{-1}	Found, µg.mL^{-1}	Recovery %
PROCTO- CINOLONE) Ointment- Medico Laboratories –Homs –Syria	50	46	99.92
(Proctoyat) Ointment Al-Hayat pharmaceutical company–Baghdad –Iraq	50	44	99.88

Table 3: Determination of Al^{3+} in dosage forms using the proposed method

Pharmaceutical preparations	Present, µg.mL^{-1}	Found, µg.mL^{-1}	Recovery%
Aciloxplus Tablet	200	193	99.97
Antespin Tablet	1000	989	99.99
Sucralfate (solution)	1000	987	99.99

Conclusions

The reagent 4-APAP showed its specificity to react only with Bi(III) and Al(III). The proposed spectrophotometric method, based on reaction of 4-APAP with the two metal ions, has been proved to be simple, rapid, precise, low cost and sensitive for Bi(III) and Al(III) using coupling reaction. Successful application of Kamlet-Taft equation to show effect of solvents of different polarities. Applying this equation correlates effects other than solvent dielectric constant to contribute their effects on electronic spectra. The procedure did not involve any critical steps; hence it can be used routinely for determination of Bi and Al in their pharmaceutical preparations with good recoveries.

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