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APPLICATION OF BENESI-HILDEBRAND ALGORITHM IN THE INVESTIGATION OF (1:2) (DRUG:REAGENT) INTERACTIONS OF PHOSPHOMOLYBDIC ACID

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ABSTRACT

The reactions between Phosphomolybdic acid (PMA) and Etamsylate (ETM) to form molybdenum blue (Mo^{5+}) (Method A) and Fluconazole (FL) to form an ion associate (Method B) were investigated in terms of stoichiometry both mathematically and chemically. Mathematical characterization of the formed adducts was done using the algorithm proposed by Benesi - Hildebrand (B-H) and its modifications. Values of both the formation constant K ($L.mol^{-1}$), and molar extinction coefficients ε ($L.mol^{-1}.cm^{-1}$) were determined using modified B-H "Rose-Drago" equation. A molar ratio of (1:2) (Drug: Reagent) was detected applying the mole ratio method applying both methods. Further investigations using B-H equation for (1:1 molar ratio) or "Rose-Drago" (for other molar ratios) confirmed the results obtained using the mole ratio method. The obtained values for ε^{AD} and K were [illegible] 10^4 $L.mol.cm^{-1}$ and 26.1×10^{11} $L.mol^{-1}$ (Method A) and 3.6×10^3 and 9.36×10^6 (Method B) respectively.

INTRODUCTION

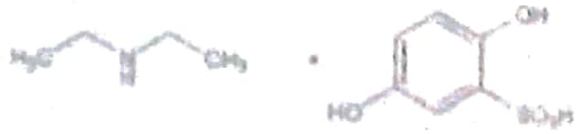
Etamsylate (ETM), chemically designated as diethyl ammonium 2,5-dihydroxy benzene sulphonic acid "Scheme 1a" is a systemic non-thrombogenic haemostatic agent that is commonly used in the treatment of hematemesis, and menorrhagia as well as post-partum hemorrhage [1]. Fluconazole (FL) "Scheme 1b", chemically known as α-(2, 4-Difluorophenyl)-α-(1*H*-1,2,4-triazol-1-ylmethyl)-1*H*-1,2,4-triazole-1 ethanol, is a commonly used antifungal. Various methods have been reported for the determination of ETM and FL either per se or in formulations and biological fluids. Among these methods, the following procedures were intensively studied, spectrophotometry [2-8], electrochemical [9-11], chemiluminescence [12, 13] and HPLC [14-17].

In the current effort, PMA "Scheme 1b" plays a dual role. While acting as an oxidizing agent which oxidizes ETM producing Mo^{+5} (Method A), it acts as a precipitating agent for FL forming an insoluble molecular complex (Method B) which can be further utilized through a redox reaction with Co(II) and EDTA to form molybdenum blue.

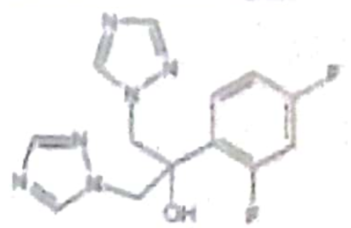
Benesi-Hildebrand method (B-H) is an algorithm used in physical chemistry for the determination of the equilibrium constant K, molar absorpitivity and stoichiometry of non-bonding interactions using optical data [18]. Assuming mainly 1:1 stoichiometry, few reports attempted the application of B-H method for the determination of equilibrium constant of 1:2 complexes, although under the conditions of $C^0_D >> C^0_A$, where C^0_D is the total drug concentration and C^0_A is the acceptor or reagent concentration; there is a possibility of formation of 1:2 stoichiometric complexes [19]. To the best of our knowledge, no reports have investigated the equilibrium constant of a redox reaction using B-H equation.

In this study, more attention is paid towards the interaction between the tested drugs and PMA in terms of mole ratio rather than the quantitative determination of the drug itself. Thus oxidation of ETM with PMA to form Molybdenum blue (Mo^{5+}) and the interaction of PMA with FL to form an ion associate complex (only the first step of the reaction was considered) were deliberated both chemically (using Mole ratio method) as well as mathematically (using B-H and the modified B-H „Rose-Drago" equations).

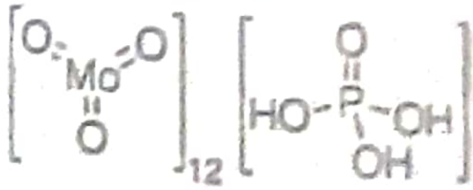
Scheme 1a: Structure of Etamsylate



Scheme 1b: Structure of Fluconazole



Scheme 1c: Structure of PMA



EXPERIMENTAL

Apparatus

A Shimadzu 260 - UV recording spectrophotometer with 10 mm quartz cell was used for all absorbance measurements.

A PSI Plot software was used to perform curve fittings.

Materials and Reagents

All chemicals were of analytical grade and doubly distilled water was used throughout all measurements. Pure Etamsylate sample was kindly supplied by Memphis Co. for Pharm. Ind., Egypt. Pure Fluconazole sample was supplied by EIPICO, 10th of Ramadan City, Egypt. Phosphomolybdic acid (PMA) (BDH), $1x10^{-2}$ and $1x10^{-3}$ M aqueous solution. Hydrochloric acid (El-Nasr Pharm. Chem., Egypt), 0.01 M aqueous solution. Cobalt (II) chloride hexahydrate; 3% w/v prepared by dissolving 3gm of $CoCl_2.6H_2O$ (Aldrich, Germany) in water and completing to 100 ml using distilled water.

5. EDTA; 4% w/v prepared by dissolving 4 gm EDTA-disodium salt (Aldrich, Germany) in water and completing to 100 ml using distilled water.

Procedure

Absorption Spectra

The electronic absorption spectrum for the reaction product was constructed as previously described in the range of 900 – 200 nm against a reagent blank [6, 7] and as shown under the following investigation of the reaction stoichiometry.

Reaction Stoichiometry

Method A

The interaction between ETM and PMA was investigated applying molar method using 1 x 10^{-3} M solutions of both ETM (V_d) and PMA (V_r) where accurately measured aliquots of the standard drug solutions (0.4 mL) were placed separately into a series of 10 ml test tubes, followed by (0.1-1.6) mL of PMA and 1 ml of 0.01 M HCl. The tubes were shaken well and heated on boiling water bath maintained at ($97\pm0.5C^0$) for 45 min. The solution was cooled to room temperature, transferred carefully and quantitatively to 10 ml volumetric flasks, diluted to the mark with ethanol and thoroughly mixed. The absorbance was measured at a 785 nm against a reagent blank similarly treated.

Method B

The stochiometry of the reaction was studied using 1 x 10^{-2} M solutions of both FL (V_d) and PMA (V_r) in presence of excess Co (II) and EDTA. A 10 mL volume of the reaction mixture contains a fixed FL concentration ($0.2x10^{-3}$ moles/10 mL) and varying concentrations of PMA ($5x10^{-5}$ – $1x10^{-3}$ moles/ 10 mL). The assigned FL concentration was added followed by 2 mL of 0.01 M HCl and then the assigned concentration of PMA and the reaction was left for 15 min. The mixture was centrifuged for 15 min., the precipitate was washed using 1 mL of distilled water, dissolved in 2.5 ml of acetone and an excess of EDTA and Co (II) solutions were added.

The absorbance was measured at 700 nm against a reagent blank.

Mathematical Depiction of the Adduct

The reaction stoichiometry was investigated applying modified Benesi – Hildebrand equations [18, 19]. Values of formation constant k ($L.mol^{-1}$) and the extinction coefficient ε ($L.mol^{-1}\ cm^{-1}$) were calculated using the appropriate B-H plots. In this itinerary, two equations were tested. The 1:1 modified Benesi – Hildebrand equation (1) [18] was tested assuming that the molar ratio is 1:1. The corresponding spectral parameters were calculated using the known Rose – Drago [19, 20] equation (2) of 1:2 adducts.

$$\frac{C_A{}^0 C_D{}^0 l}{A} = \frac{1}{K\varepsilon} + \frac{C_A{}^0 + C_D{}^0}{\varepsilon} \qquad (1)$$

$$\frac{C_D{}^{0^2} C_A{}^0}{A} = \frac{1}{K\varepsilon} + \frac{1}{\varepsilon}.C_D{}^0 (C_A{}^0 + 4\,C_D{}^0) \qquad (2)$$

Where, C^0_A and C^0_D are the initial concentrations of reagent and drug respectively (calculated on the basis of 10 mL volume), while A is the absorbance at the assigned wavelength and is the molar absorpitivity at the specified wavelength.

RESULTS AND DISCUSSION

Reaction Stoichiometry

The electronic spectrum of the reaction of ETM and PMA is shown in (Fig.1). The equal mole ratio method was used to determine the reaction mole ratio of PMA and ETM, which is shown in (Fig. 2), where the concentration of the drug (C_D) was kept fixed, whereas that of PMA (C_A) was changed over a wide range. The drug/ reagent molar ratio (C_D: C_A) obtained for Method A varies over the range of 1.00:0.25 – 1.00:4.00, while for Method B, the range was 1.00:0.25 – 1.00:5.00.

As can be seen (Fig. 2), the molar ratio for the reaction between ETM and PMA (Method A) was 1:2 (Drug: Reagent). As also shown in this figure, the absorbance would increase as the concentration of PMA increases, the issue which is noticeable in some redox reactions making the process of locating an accurate mole ratio between drug and reagent an intricate task.

A similar behavior was observed using PMA as an ion pairing reagent for FL (Method B), where the molar ratio was (1:2) (D:R) with a slight increase in absorbance as the mole fraction of PMA increases. In a trial to overcome this issue, mathematical algorithms proposed by Benesi and Hildebrand and the one proposed by Rose-Drago [18-20] were applied making usage of the optical results obtained from the mole ratio method.

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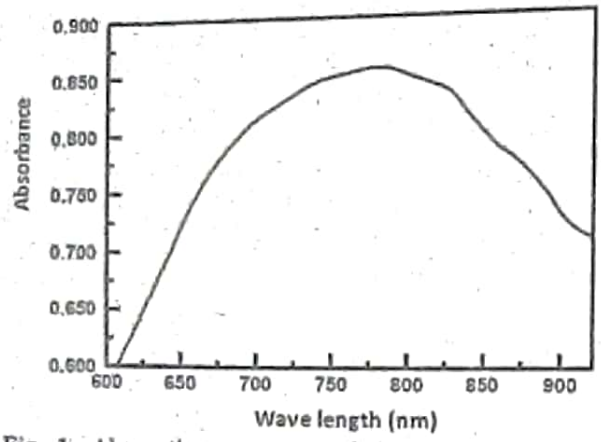

Fig. 1: Absorption spectrum of the reaction product resulting from reaction of 18 μgml^{-1} ETM with PMA.

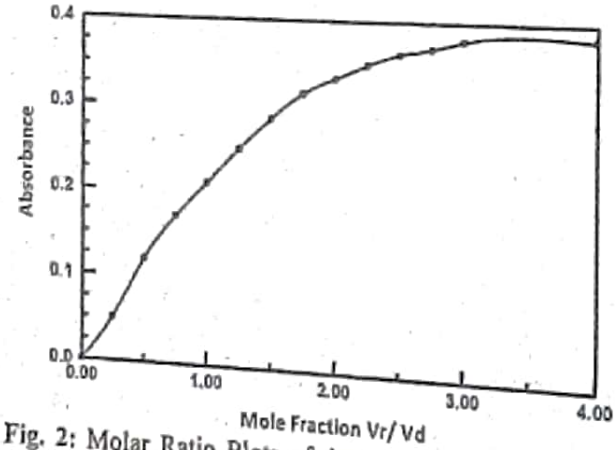

Fig. 2: Molar Ratio Plots of the Reaction between 1x 10^{-3} M PMA and 1x 10^{-3} M ETM.

Benesi-Hildebrand Investigation

The 1:1 modified Benesi – Hildebrand equation [18, 19] (1) was tested assuming that the molar ratio is 1:1. On plotting values of $C^0_A C^0_D / A$ versus $(C^0_A + C^0_D)$, a set of scattered points was obtained, as shown in case of oxidation of ETM with PMA (Fig. 3), indicating that the molar ration is not 1:1.

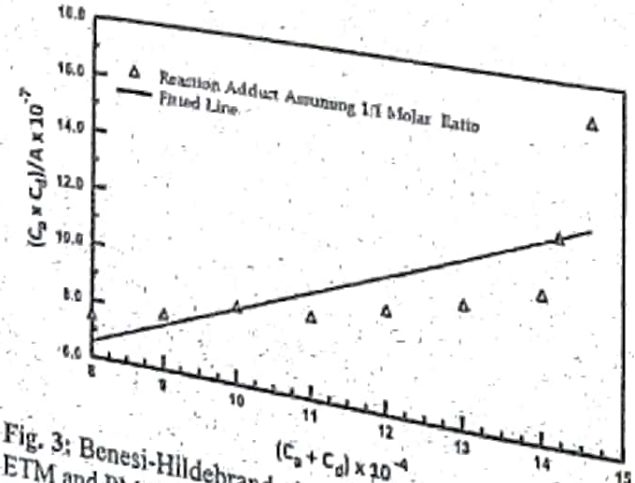

Fig. 3: Benesi-Hildebrand plot for the reaction between ETM and PMA assuming 1:1 molar ratio.

The corresponding spectral parameters were calculated using the known [19, 20] equation (2) of 1:2 adducts. On plotting values of $(C^0_D)^2 C^0_A/A$ versus $C^0_D (4C^0_D + C^0_A)$, straight lines were obtained with a slope of $1/\varepsilon^{AD}$ and intercept of $1/ k\varepsilon^{AD}$ as shown in (Fig. 4.) and (Fig. 5.) Such results assure the finding that the molar ratio is 1:2 (Drug: Reagent).

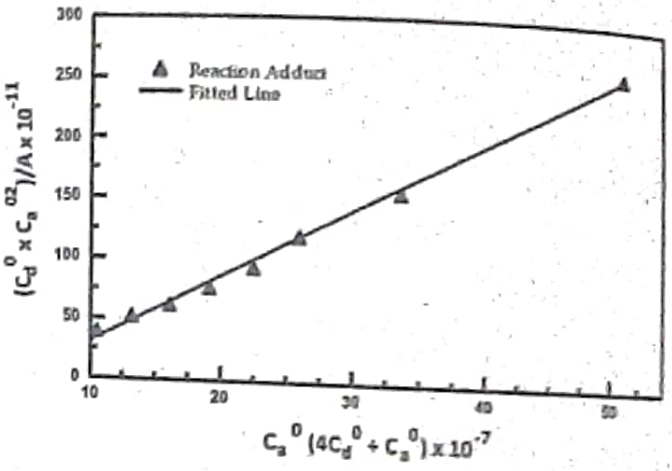

Fig. 4: Rose-Drago plot assuming 1:2 (D:R) molar ratio for the reaction of ETM and PMA in 10 mL total sample.

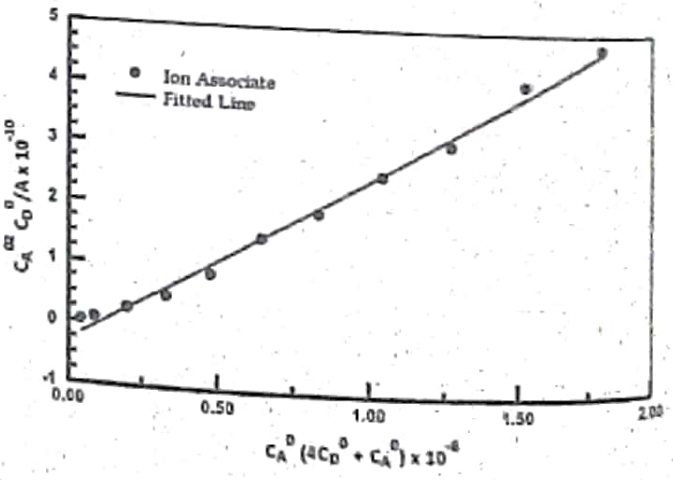

Fig. 5: Rose-Drago plot for 1:2 (D:R) molar ratio for the reaction of FL and PMA in 10 mL total sample.

From Fig. 1, 4 and 5, values of ε^{AD} and K were calculated according to the slope and the intercept of the linear relationship "y=a+bx" obtained by linear fitting of the data. The calculated formation constants and molar absorpitivities are summerized in Table 1. However, it should be noticed that ε^{AD} which is the molar absorpitivity of the complex itself should not be confused with any stoichiometric value calculated with reference to the amount of any analyte being determined. The latter is best described as Beer"s law value while the former is a Benesi-Hildebrand"s value. As shown in Table 1; the obtained values of ε and K are high enough to confirm the expected high stability of the formed adduct.

Table I: Optical Parameters determined using Benesi-Hildebrand model compared to Beer"s law:

Parameter	Method A	Method B
Molar Absorpitivity (L.mol $^{-1}$ .cm $^{-1}$) –Beer's Law	1.25×10^4	1.307×10^3
ε^{AD} (L.mol $^{-1}$ .cm $^{-1}$) -B-H Value	1.86×10^6	3.6×10^3
K (L.mol $^{-1}$) -B-H Value	26.1×10^{11}	9.36×10^6

CONCLUSION

Spectrophotometric characterization of the reaction between etamsylate and PMA (Method A) and PMA with fluconazole (Method B) has been described. Both chemical and mathematical characterization (using B-H equation and Rose-Drago model) of the reaction product reveals that PMA forms 1:2 (Drug: Reagent) adduct with both etamsylate and fluconazole in aqueous and organic media. In addition, experimental evidences shows the formation of 1:2 complexes but not 1:1 complexes under the mentioned reaction conditions. The formation constants for both reactions were calculated using Rose-Drago model and their relatively high values indicate the stability of the formed reaction product.

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تطبيق نظام الحلول الحسابية لبنس هيلدبراند في فحص تفاعلات حمض الفوسفوموليبديك نسبة العقار: الكاشف (1 : 2)

مروة سعيد العزازي – وفاء السيد حسن
قسم الكيمياء التحليلية – كلية الصيدلة – جامعة الزقازيق
الزقازيق – مصر – قسم الكيمياء التحليلية – كلية الصيدلة

في هذا البحث تم تفاعل الكاشف وهو حمض الفوسفوموليبديك مع عقار الايثامسيلات لتكوين أزرق الموليبدنيوم (الطريق الأولى) وعقار الفلوكونازول لتكوين المتركب الايوني (الطريقة الثانية) وقد تمت دراسة الحسابات الكيميائية رياضيا وكيميائيا.

لقد تم حسابيا استخدام نظام الحلول الحسابية المقترحة لنسب هيلدبراند قد تم تعيين قيم ثوابت التكوين ومعامل الامتصاص الجزيئي باستخدام هذه المعادلات المقترحة. وكيميائيا تم تحديد النسبة الجزئية (1 : 2) من العقار الكاشف باستخدام طريقة النسبة الجزئية.