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Spectrophotometric determination of gliclazide in pharmaceuticals and biological fluids through ternary complex formation with eosin and palladium (II)

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Abstract

A simple and sensitive spectrophotometric method has been developed for the determination of gliclazide (GLZ) in pharmaceutical formulations and biological fluids. The proposed method is based upon the formation of a ternary complex between palladium (II), eosin and GLZ in the presence of methyl cellulose as a surfactant and acetate buffer of pH 4.5. The ternary complex showed an absorption maximum at 550 nm. The solution of ternary complex obeyed Beer's law over the concentration range of 0.5–4 μg ml^{-1} with minimum detectability (S/N = 2) of 0.05 μg ml^{-1} (1.545×10^{-7} M). The different experimental parameters affecting the development and stability of the color were carefully studied and optimized. The proposed method was successfully applied to the analysis of commercial tablets. The results obtained were in good agreement with those obtained using the official or reference spectrophotometric method. The proposed method was further applied to spiked human urine and plasma, the percentage recoveries were 97.84 ± 0.72 and 97.43 ± 0.83, respectively, (n = 4). A proposal of the reaction pathway was presented.

Keywords: Spectrophotometry; Gliclazide; Ternary complex; Eosin; Palladium chloride; Pharmaceutical analysis and biological fluids

1. Introduction

Gliclazide (GLZ) *N*-[[(Hexahydrocylopenta[c]pyrrol-2(1H)-yl)amino]carbony]-4-methylbenzene sulfonamide is widely used as antidiabetic [1]. It is orally administered in the treatment of type 2 diabetes mellitus and has a duration of action of 12 h or more. As its effects are less prolonged than those of chlorpropamide or glibenclamide, it may be more suitable for elderly patients, who are prone to hypoglycemia with longer-acting sulfonylureas.

Several methods have been published for the determination of GLZ, either per se or in pharmaceutical preparations and biological fluids. These methods include: non-aqueous titration [2], spectrophotometry [3,4], radioimmunoassay [5], GLC [6,7], TLC [8] and HPLC [9–15]. All these methods are either insufficiently sensitive [2–4] or tedious and require highly sophisticated and dedicated instrumentation [9–15]. This led us to study its reaction through ternary complex formation with eosin and palladium in an attempt to develop simple and sensitive spectrophotometric method for the determination of GLZ in pharmaceutical preparations and biological fluids.

Work from our laboratory described the use of 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) as derivatizing agent for GLZ and measuring the product either spectrophotometrically at 400 nm or spectrofluorimetrically at 470 nm after excitation at 400 nm [16].

However, the proposed method is characterized by being more sensitive, as the concentration range is 0.5–4 μg ml^{-1} with a detection limit of 0.05 μg ml^{-1} compared with 2–20 μg ml^{-1} with a detection limit of 0.2 μg ml^{-1} for the NBD-Cl method. In addition, the proposed method could be successfully applied to spiked biological samples. Moreover, the reagents used in the proposed method are stable for at least 2 weeks while NBD-Cl must be freshly prepared. The proposed method is considered as stability indicating method, since the side of complex formation is expected to be the side of degradation (hydrolysis).

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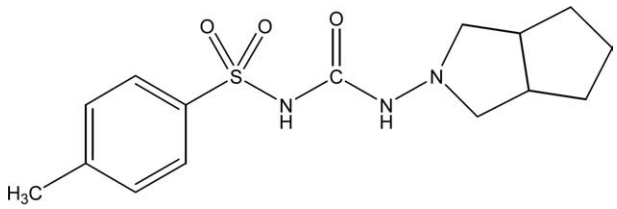
Ternary complex formation had been used for the determination of Palladium (Pd (II)) via 1,10 phenanthroline as a cationic component and eosin as an anionic counter ion [17]. On the same basis, Fujita et al [18] determined a group of drugs by forming ternary complex with Pd (II) and eosin. In their studies nine cations have been tried, Pd (II) proved to be the only effective metal ion.

Color reactions of various drugs in aqueous media were investigated utilizing the ternary complex formation such as chlorpromazine, thiamine, lincomycin, ofloxacin and theophylline [18], ciprofloxacin and norfloxacin [19] and astemizole, terfenadine and flunarizine hydrochloride [20].



Structural Formula of gliclazide

2. Experimental

2.1. Apparatus

The spectrophotometric measurements were established using Shimadzu UV–visible 1601 recording spectrophotometer (P/N 206-67001). Recording range, 0–1.0; wavelength, 550 nm.

2.2. Materials and reagents

All reagents and solvents were of analytical reagent grade.

1. GLZ pure sample was kindly provided by Pharco. Pharmaceutical Co., Alexandria, Egypt. Its purity was 99%.
2. Tablets containing 80 mg of GLZ each (Diamicron tablets, Batch # 1G 392, product of Servier Egypt Ind., Cairo, Egypt) and Glipicrone tablets, Batch # 203904, product of Amriya Pharm. Ind. Alexandria, Egypt. They were obtained from commercial sources in the local market.
3. Eosin (Merck, Darmstadt, Germany) was prepared as 2.0×10^{-3} M, aqueous solution. The solution is stable for 2 weeks.
4. $PdCl_2$ (Sigma, Milwaukee, WI, USA) was prepared as 2×10^{-3} M solution by dissolving about 35.5 mg of $PdCl_2$ in 1 ml of hydrochloric acid, with the aid of heat, followed by the addition of 50 ml of boiled water and diluting to 100 ml with distilled water. This solution is stable for 2 weeks.
5. Methyl cellulose (MC) (Prolabo, France) 1500 cP, 0.5% w/v aqueous solution, prepared by dissolving the appropriate amount in hot water (80 °C) with stirring for 10 min, then chilling to 5 °C for 30 min.
6. Acetate buffer (pH values of 4.5 and 3.9), prepared by mixing 0.2 M acetic acid solution and 0.2 M sodium acetate solution, the pH has to be checked periodically [21].
7. Plasma was kindly provided by Mansoura University Hospital, and kept frozen untill assay after gentle thawing.
8. Urine sample was obtained from healthy volunteers (male around 40 years old).
9. Methanol, hydrochloric acid and chloroform (Merck, Darmstadt, Germany).

2.3. Standard solutions

A stock solution was prepared by dissolving 20.0 mg of GLZ in 100 ml of methanol and was further diluted with the same solvent as appropriate. The standard solutions were stable for 7 days when kept in the refrigerator.

2.4. Procedures

2.4.1. Calibration Graph. Transfer aliquot volumes of GLZ standard solution into a series of 10 ml volumetric flasks. Add 1.5 ml of 0.5% MC solution, add 2 ml of acetate buffer pH (4.5) followed by 1.0 ± 0.1 ml of eosin solution and 0.7 ± 0.1 ml of $PdCl_2$ solution. Heat at 60 °C ± 2 °C for 20 min in a thermostatically-controlled water-bath, then cool for 5 min at 25 °C. Complete to the mark with distilled water. Measure the absorbance of the solution at 550 nm against similarly prepared eosin—$PdCl_2$ solution (blank solution). Plot the measured absorbance vs. the final concentration to get the calibration curve. Alternatively, derive the corresponding regression equation.

2.4.2. Procedure for tablets. Weigh and pulverize 20 tablets. Transfer a weighed quantity of the powder equivalent to 20 mg of GLZ into a small conical flask, extract with 3 × 30 ml of methanol. Filter the extract into 100 ml volumetric flask. Wash the conical flask with few milliters of methanol. Pass the washings into the same conical flask and complete to the mark with the same solvent. Transfer aliquot volumes covering the working concentration range over 0.5–4 μg ml^{-1} into 10 ml volumetric flasks. Proceed as described under "Calibration Graph". Determine the nominal content of the tablets either from the calibration curve or using the corresponding regression equation.

2.4.3. Procedure for spiked urine. Transfer 5 ml of urine into a 125 ml separating funnel. Spike with increasing quantities of GLZ to give a final drug concentration cited in Table 3. Add 1 ml of acetate buffer (pH 3.9) and shake well. Extract with 3 × 5 ml of chloroform, and then pass the chloroform layer over anhydrous sodium sulfate. Evaporate the extract under reduced pressure till dryness. Dissolve the residue in 5 ml of methanol and then proceed as described under "Calibration Graph". Determine the nominal content of the drug from the corresponding regression equation.

2.4.4. Procedure for spiked plasma. Transfer 1 ml of plasma into a 125 ml separating funnel. Spike with increasing quantities of GLZ to give a final drug concentration cited in Table 3. Add 1 ml of acetate buffer (pH 3.9) and shake well. Extract with 3 × 5 ml of chloroform, then pass the chloroform layer over anhydrous sodium sulfate. Evaporate the extract under reduced pressure till dryness. Dissolve the residue in 5 ml of methanol and then proceed as described under "Calibration Graph". Determine the nominal content of the drug from the corresponding regression equation.

3. Results and discussion

The main purpose of this study was to establish a simple spectrophotometric method for the determination of GLZ in pure form, pharmaceutical preparations and biological fluids. The proposed method is based on ternary complex formation with eosin and Pd (II). The ternary complex formed between the metal ion: electronegative ligand and organic base often have higher values of molar extinction coefficient than binary complexes of the same components. The formation of ternary complexes improves not only the sensitivity of the method but also the selectivity as well.

In the present study, GLZ was found to form a ternary complex with each of eosin and Pd (II) in the presence of MC and at pH 4.5 producing a red color with maximum absorbance value at 550 nm (Fig. 1).

3.1. Optimization of the experimental conditions

The spectrophotometric properties of the colored product as well as the different experimental parameters affecting the color development and its stability were carefully studied and optimized. Such factors were changed individually while the others were kept constant. The factors include pH, type of buffer, type of metal cation, temperature, time of heating, effect of different sensitizers, different surfactants, concentration of surfactants, and concentrations of eosin and Pd (II).

The influence of pH on the absorbance value of the ternary complex was studied at 550 nm. The absorbance of the drug–Pd (II)–eosin complex solution was investigated over the pH range 3.6–5.6. Maximum absorbance value was achieved at pH 4.5, using 2 ml of acetate buffer. Other buffers having the same pH value such as phosphate and Britton Robinson buffers were tried and compared with 0.2 M acetate buffer. Acetate buffer was found to be superior to phosphate and Britton Robinson buffers having the same pH value since the net absorbance value was highest in case of acetate buffer (Scheme 1).

The effect of eosin and Pd (II) concentrations on the absorbance of the ternary complex was studied keeping the concentration of the drug and Pd (II) constant and varying eosin concentration, it was found that increasing the volume of eosin (2×10^{-3} M) resulted in a subsequence increase in the absorbance value of the ternary complex up to 0.75 ml,

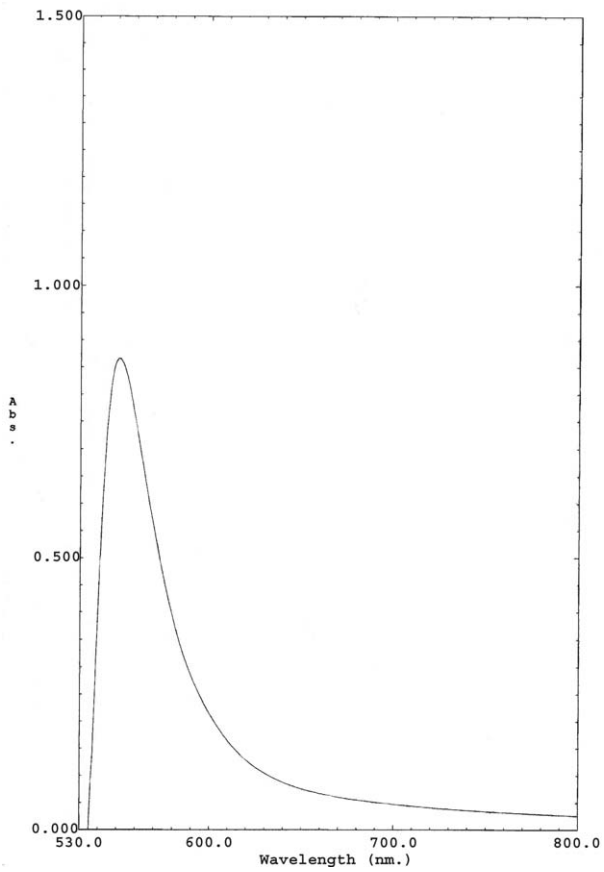


Fig. 1. Absorption spectrum of the ternary complex of GLZ (4 μg ml^{-1}) with eosin and Pd (II) at pH 4.5.

and remained constant up to 1.1 ml therefore, 1.0 ± 0.1 ml which resulted in a final concentration of 2×10^{-4} M was used as the optimum concentration of eosin (Fig. 2).

The effect of volume of Pd (II) on the absorbance value of the ternary complex was also studied keeping the concentration of the drug and eosin constant. It was observed that increasing the volume of Pd (II) (2×10^{-3} M) would result in a gradual increase in the absorbance of the ternary complex up to 0.6 ml and remained constant up to 0.8 ml after which the absorbance of the complex began to decrease. Thus, 0.7 ± 0.1 ml of 2×10^{-3} M of Pd (II) which resulted in a final concentration of 1.4×10^{-4} M was used through out this approach (Fig. 3).

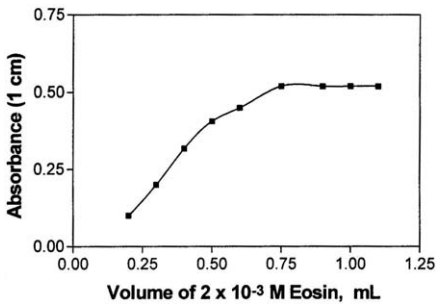


Fig. 2. Effect of volume of 2×10^{-3} M eosin, ml on the absorbance value of the ternary complex of GLZ (2.5 μg ml^{-1}) at pH 4.5.

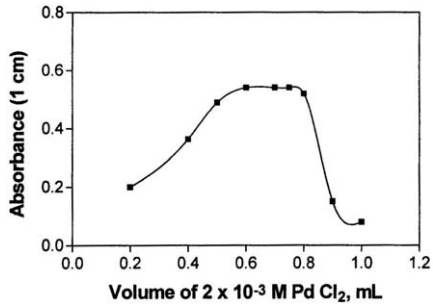


Fig. 3. Effect of volume of 2×10^{-3} M $PdCl_2$, ml on the absorbance value of the ternary complex of GLZ (2.5 μg ml^{-1}) at pH 4.5.

In order to examine the effect of temperature and heating time on the formation rate and on the absorbance of the drug–Pd (II)–eosin ternary complex, the experiment was carried out at different temperatures settings (room temperature, 40, 50, 60 and 70 °C) using a thermostated water-bath for periods raging from 10 to 40 min. Maximum and constant absorbance value was obtained at 60 °C after 20 min (Fig. 4). The solution was cooled under tap water for 5 min to room temperature with agitation before measuring the absorbance to solubilize jelly-like aggregates formed upon heating at 60 °C. As reported by other investigators, the reproducibility was somewhat poor on using hot solution [22]. The ternary complex formed remains stable for more than 2 h.

The effect of surfactants on the absorbance of the ternary complex was examined using various dispersing agents, such as cetylpyridinium chloride (cationic), sodium lauryl sulfate (anionic) and MC 1500 cP (non-ionic). Among the surfactants studied, best results were obtained in the presence of

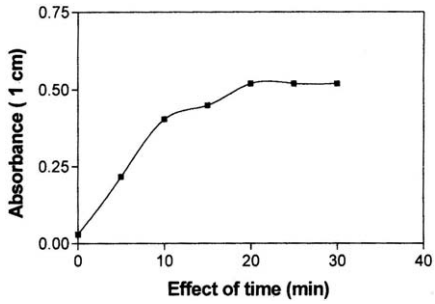


Fig. 4. Effect of heating time (min) on the absorbance value of the ternary complex of GLZ (2.5 μg ml^{-1}) at pH 4.5.

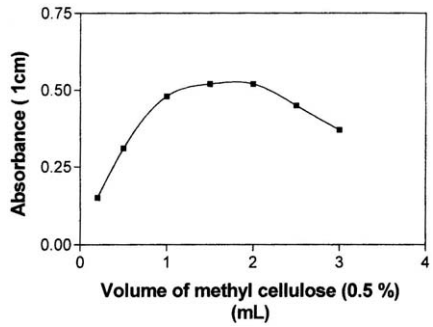


Fig. 5. Effect of volume of MC (1500 cP) (0.5%) (ml) on the absorbance value of the ternary complex of GLZ (2.5 μg ml^{-1}) at pH 4.5.

MC (1500 cP). Maximum and constant absorbance was obtained using 1.5 ml of 0.5% w/v MC solution (Fig. 5).

When the non-ionic surfactant MC was used, prior extraction steps were unnecessary. The addition of surfactants to solubilize and stabilize the ternary complex had been previously reported [18]. Cationic surfactants such as cetylpyridinium chloride decreased the color of the formed complex probably due to the formation of an ion-pair complex between eosin and the cationic surfactant. MC, which is a non-ionic water-soluble polymeric surfactant, was reported to be the best dispersing agent with respect to sensitivity [18], in addition, it increases the stability of the complex and prevent its precipitation, accordingly, MC was used in this study. The acid dissociation properties of eosin in the presence of MC were determined spectrophotometrically at ionic strength of 0.1 M at 20 ± 0.1 °C [23,24].

Depending on the pH of the solution, eosin can exist in any of the following forms:

$$H_3R^+ \underset{-H^+}{\overset{K_{a1}}{\rightleftharpoons}} H_2R \underset{-H^+}{\overset{K_{a2}}{\rightleftharpoons}} HR^- \underset{-H^+}{\overset{K_{a3}}{\rightleftharpoons}} R^{2-}$$

where R denotes the anionic part of eosin. In this study, the pK_{a1}, pK_{a2}, pK_{a3}, in the presence of MC were 2.10, 2.85 and 4.95, respectively. At pH 4.3 about 80% of eosin was found to be in the form HR^- [25].

Different sensitizers were also tried such as, fluorescein, rhodamine 6 G and quinine HCl. Eosin was found to be the best one, since it gave the highest and constant absorbance value of the complex. In the same manner various metals were studied to choose the most suitable one for formation of ternary complex, e.g. $FeCl_3$, $CuSO_4$, Pb $(AC)_2$ and $PdCl_2$, the latter was found to be the most suitable metal since it gave the highest absorbance reading.

3.2. Analytical performance

The absorbance–concentration plot was found to be linear over the range of 0.5–4 μg ml^{-1} with minimum detectability (S/N = 2) of 0.05 μg ml^{-1} (1.545×10^{-7} M). Linear regression analysis of the data gave the following equation:

$$A = -5.15 \times 10^{-3} + 0.213C$$
$$(r = 0.9998)$$

where A is the absorbance in 1-cm cell and C is the concentration of the drug in μg ml^{-1}.

The limit of quantification (LOQ), which is the lowest concentration that can be measured was found to be 0.5 μg ml^{-1} adopting the proposed method. It was determined by taking the concentration, which gives a reliable absorbance reading (0.105 A unit), below which the calibration graph was not linear. The limit of detection (LOD) was determined by establishing the minimum level at which the analyte can be reliably detected and was found to be (S/N = 2) of 0.05 μg ml^{-1}.

3.3. Validation of the method

The method was tested for linearity, selectivity and precision. By using the above spectrophotometric procedure, linear regression equation was obtained. The regression plots showed that there was a linear dependence of the absorbance values on the concentration of the drug over the range of 0.5–4 μg ml^{-1}. The validity of the method was evaluated by statistical evaluation of the regression lines. It was found that the standard deviation of the residuals ($S_{y/x}$) is 4.88×10^{-3}, the standard deviation of the intercept (S_a) is 7.063×10^{-3} and standard deviation of the slope (S_b) is 1.554×10^{-3}. The small values of the figures point out to the low scattering of the points of the calibration curve.

The precision of the proposed method was evaluated by analyzing standard solutions of GLZ. The results obtained by the proposed method were compared with those given by the official method [2].

Statistical analysis [26] of the results obtained by the proposed and official methods using the Student's *t*-test and variance ratio *F*-test, showed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively (Table 1).

The selectivity of the method was investigated by observing any interference encountered from the excipients of the tablets. It was shown that these excipients did not interfere with the proposed method (Table 2).

Table 1
Application of the proposed and reference methods to the determination of gliclazide in pure form

Parameters	Spectrophotometric method	Official method [2]
1. No.of experiments	**7**	3
2. Mean found(%) ± S.D.	99.83 ± 0.98	100.52 ± 1.04
3. Variance	0.96	1.082
4. Student's *t*-value	0.99 (2.31)	
5. Variance ratio *F*-test	1.13 (5.14)	

N.B. Figures between parentheses are the tabulated *t* and *F* values, respectively, at $P = 0.05$ [26].

3.4. Pharmaceutical applications

The proposed method was further applied to the determination of GLZ in its tablets. Common tablets excipients such as talc, lactose, starch, avisil, gelatin and magnesium stearate did not interfere with the assay. The results obtained were compared with those giving using a reference method [3]. Statistical analysis [26] of the results using Student's *t*-test and variance ratio *F*-test, revealed no significant difference between the two methods at the 95% confidence level regarding accuracy and precision, respectively. The results obtained are abridged in Table 2.

3.5. Analysis of biological fluids

The high sensitivity of the proposed method allowed the determination of GLZ in biological fluids. The proposed method was further applied to the in-vitro determination of GLZ in spiked human urine and plasma.

GLZ is readily absorbed from the gastro-intestinal tract. The half-life time is about 10–12 h. GLZ is extensively metabolized without significant hypoglycemic activity. The usual initial dose is 40–80 mg daily, gradually increased if necessary, up to 320 mg daily. Doses of more than 160 mg daily are given in two divided doses [1]. Following oral ingestion of a single 80 mg GLZ dose gives a final plasma concentration of about 1.6 μg ml^{-1}. This value lies within the working concentration range of the proposed method, thus it could be successfully applied to the determination of GLZ in spiked human urine and plasma over the specific concentration range. The results are abridged in Table 3. The mean percentage recoveries for GLZ in spiked urine and plasma are 97.84 ± 0.72 and 97.43 ± 0.83, respectively ($n = 4$). The method involved extraction of the drug using chloroform. The extraction procedure described by Wang et al [13] was adopted.

3.6. Precision

The within-day precision was evaluated through replicate analysis of urine and plasma samples spiked with different

Table 2
Application of the proposed methods to the determination of gliclazide in dosage forms

Preparations	Recovery (%)	
	Proposed method	Reference method [3]
1. *Diamacron tablets*[a] (GLZ 80 mg/tablet)		
Mean found (%) ± S.D.	100.89 ± 0.77	99.65 ± 1.35
t-value	1.69	
F-value	3.08	
2. *Glipicrone tablets*[b] (GLZ 80 mg/tablet)		
Mean found (%) ± S.D.	99.84 ± 1.19	100.12 ± 1.21
t-value	0.32	
F-value	1.03	

N.B. The tabulated values of *t* and *F* are (2.45) and (6.94), respectively, at $P = 0.05$ [26]

[a] Product of Servier Egypt Industries, Cairo, Egypt.

[b] Product of Amriya Pharm. Industries, Alexandria, Egypt.

Table 3
Application of the proposed methods to the determination of gliclazide in spiked urine and plasma

Sample	Amount added (μg ml^{-1})	Amount found (μg ml^{-1})	Recovery (%)
1 Urine (intra-day precision)	1.0	0.985	98.50
	2.0	1.955	97.75
	3.0	2.906	96.87
	4.0	3.929	98.23
Mean ± S.D.			97.84 ± 0.72
2 a-Plasma (intra-day precision)	1.0	0.969	96.90
	2.0	1.954	97.70
	3.0	2.897	96.57
	4.0	3.934	98.35
Mean ± S.D.			97.43 ± 0.83
2 b-Plasma (inter-day precision)	4.0	3.779	94.48
	4.0	3.892	97.30
	4.0	3.835	95.88
	4.0	3.910	97.75
Mean ± S.D.			96.35 ± 1.48

N.B. Each result is the average of three separate determinations.

concentrations of the drug. The percentage recoveries based on the average of four separate determinations were 97.84 ± 0.72 and 97.43 ± 0.83, for urine and plasma samples, respectively, thus indicating the high precision of the method.

The inter-day precision was also evaluated through replicate analysis of plasma samples spiked with 4 μg ml^{-1} of drug on four successive days. The percentage recoveries based on the average of four separate determinations were 96.35 ± 1.48. The results are shown in Table 3.

3.7. Mechanism of the reaction

The stoichiometry of the reaction was studied adopting the limiting logarithmic method [27]. The absorbance of the reaction product was alternatively measured in the presence of excess of $PdCl_2$ and GLZ. A plot of log absorbance vs. log [$PdCl_2$] and log [GLZ] gave straight lines, the values of the slopes are 0.77 and 1.01, respectively, (Fig. 6). Hence, it is concluded that, the molar reactivity of the reaction is 0.77/1.01, i.e. the reaction proceeds in the ratio of 1:1, confirming that one molecule of the drug denses with one molecule of $PdCl_2$ (Fig. 6). The drug reacts via the amide group with the Pd (II) ion in the presence of eosin. Based on the obtained molar reactivity, the reaction pathway is proposed to proceed as follows:

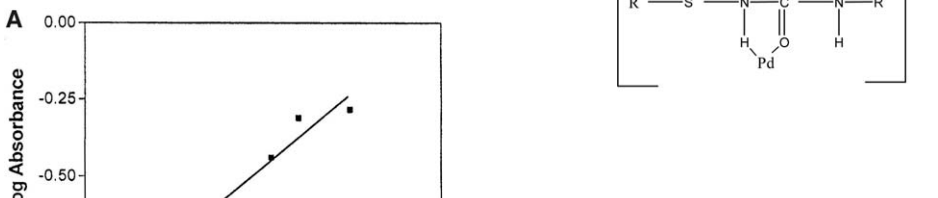
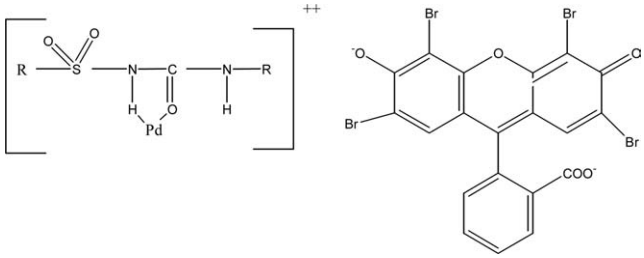


Fig. 6. Limiting logarithmic plots for the molar ratio. (A) Log *A* vs. Log [$PdCl_2$] (B) Log *A* vs. log [GLZ]



Scheme 1. The ternary complex of GLZ–eosin–Pd (II).

4. Conclusion

The proposed method has the advantages of being simple, sensitive and suitable for routine analysis in control laboratories. The proposed method is considered as stability indicating method, since the side of complex formation is expected to be the side of degradation (hydrolysis). The ternary complex formed did not require prior extraction procedure and have the advantages of being suitable for the determination of GLZ in spiked human urine and plasma with minimum detection limit comparable to reported values. Moreover, it could be applied to the determination of different pharmaceutical dosage forms.

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