Research Article

Spectrophotometric Determination of Oxybutynin Hydrochloride via Charge- Transfer Complexation Reaction

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ABSTRACT

Two simple, accurate and reproducible spectrophotometric methods were developed for the quantitative estimation of oxybutynin hydrochloride in pure form and in pharmaceutical preparation. The methods were based on the charge transfer complex formation of oxybutynin as *n*-electron donor with two π-electron acceptors: 2,3-dichloro-5,6-dicayno-*p*-benzoquinone (DDQ) (DDQ method) and 2,5-dichloro-3,6-dihydroxy-*p*-benzoquinone (*p*-chloranilic acid) (*p*-chloranilic acid method) in acetonitrile. Different variables affecting the reactions were investigated and optimized**.** Under the optimum reaction conditions, linear relationship with good correlation coefficients (0.9998 - 0.9999) was found between the absorbances at 457 nm and 520 nm and the concentrations of oxybutynin over the concentration ranges of 20 - 80 µg ml^{-1} and 30 - 160 µg ml^{-1}, for DDQ and *p*-chloranilic acid methods, respectively. The proposed methods were validated in accordance with ICH guidelines and were applied successfully to pharmaceutical formulation. The stoichiometric relationship determined by Job's continuous variation method was found to be 1:1 (drug: reagent) for both methods. Statistical comparison of the results obtained by applying the proposed methods and the reference method was carried out and revealed no significant difference between the results. Therefore, the charge transfer approach using DDQ and *p*-chloranilic acid can be applied successfully for the determination of oxybutynin in tablets in quality control laboratories.

Keywords: Oxybutynin hydrochloride; DDQ; *p*-chloranilic acid; spectrophotometry.

1. INTRODUCTION

Oxybutynin hydrochloride (Fig. 1) is chemically designated as 4-(diethyl amino)-2-butynyl (±)-α-phenylcyclohexaneglycolate hydrochloride[1]. It is an anti-cholinergic and antispasmodic agent which is commonly indicated for the treatment of overactive bladder with symptoms of urge urinary incontinence and frequency[2]. It reduces uninhibited bladder contractions but although it may be of use in diurnal enuresis, it is rarely of benefit in nocturnal enuresis alone[3]. Literature survey revealed that few methods have been performed for the analysis of oxybutynin hydrochloride such as spectrophotometry[4-9], spectrofluorimetry[6], voltammetry[2], HPTLC[9,10] and HPLC [8,11,12,13]. On the other hand, no colorimetric method based on charge transfer complexation was reported for the determination of the cited drug.

Visible spectrophotometric methods represent the most convenient analytical technique in most quality control laboratories because of their selectivity. In addition, they are easier, less expensive, and less time consuming compared with many other methods.

The aim of the following investigation was to establish sensitive, simple and precise visible spectrophotometric methods for the determination of oxybutynin in pharmaceutical formulation with no need for any expensive or sophisticated instruments.

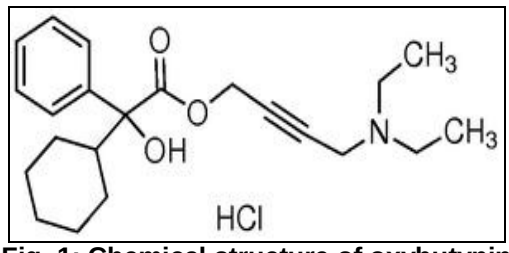


Fig. 1: Chemical structure of oxybutynin hydrochloride

2. Experimental

2.1. Apparatus

A double beam Schimadzu Ultraviolet/Visible recording spectrophotometer 1600/Japan, connected to an IBM compatible computer and supported with UV Probe software version 2.21 was used for spectrophotometric measurements.

2.2. Chemicals and reagents

Oxybutynin hydrochloride pure sample (its purity was analyzed and found to be 100.05±0.703, by applying reference method[14] was kindly supplied by ADWIA Co. S.A.E., 10th of Ramadan City, Egypt. Uripan® tablets (labelled to contain 5 mg of oxybutynin hydrochloride per tablet, batch No. 120744 and121058) were manufactured by ADWIA Co. S.A.E., 10th of Ramadan City, Egypt and were purchased from local market. DDQ and *p*-chloranilic acid (Sigma-Aldrich, Germany) were freshly prepared as 2 x 10^{-2} M and 2.38 x 10^{-3} M solutions, respectively, in acetonitrile (HPLC grade, Sigma-Aldrich, Germany). Sodium sulphate anhydrous, chloroform and sodium carbonate (prepared as an aqueous solution 10% w/v) were obtained from EL-Nasr Pharmaceutical Chemicals Co., Egypt.

2.3. Stock solutions

Oxybutynin base stock standard solution

Oxybutynin hydrochloride (equivalent to 200 mg oxybutynin base) was dissolved in distilled water (20 ml) and then transferred quantitatively into a 250 ml separating funnel. Ten ml of sodium carbonate solution (10% w/v) were added and the liberated base was extracted with chloroform (10 ml x 5).The combined chloroform extracts were filtered through anhydrous sodium sulphate into a 100 ml volumetric flask and then the volume was completed to the mark with chloroform to produce a stock solution of concentration (2 mg ml^{-1}).

Oxybutynin base working standard solution

Different aliquots (10 ml and 20 ml) of oxybutynin base stock standard solution were evaporated to dryness, separately, on a water bath. The residue was dissolved in acetonitrile (10 ml) and then quantitatively transferred into two separate 100 ml volumetric flasks. The volume was completed to the mark with the same solvent in order to obtain working standard solutions of concentrations 200 µg ml^{-1}(DDQ method) and 400 µg ml^{-1} (*p*-chloranilic acid method).

2.4. General procedures and linearity

DDQ method

Different aliquots of oxybutynin base working standard solution (200 µg ml^{-1}) containing (200 – 800 µg) of oxybutynin base were transferred quantitatively into a series of 10 ml volumetric flasks and 3ml of DDQ (2 x 10^{-2} M) solution were added to each flask. After 10 min, each flask was completed to volume with acetonitrile and the absorbance was measured at 457 nm against reagent blank. A calibration curve relating the absorbances at 457 nm and the corresponding concentrations of oxybutynin was constructed and the regression equation was computed.

p-Chloranilic acid method

Into a series of 10 ml volumetric flasks, various aliquots of oxybutynin base working standard solution (400 µg ml^{-1}) containing (300 – 1600 µg) of oxybutynin were transferred then allowed to react with 4 ml of *p*-chloranilic acid solution (2.38 x 10^{-3} M) for 5 minutes and the volume was completed with acetonitrile. The absorbance was measured at 520 nm against reagent blank. A calibration curve relating the absorbances at 520 nm and the corresponding concentrations of oxybutynin was constructed and the regression equation was computed.

2.5. Analysis of pharmaceutical formulation

Twenty Uripan® tablets were accurately weighed and finely powdered. An accurate weight of the powdered tablets equivalent to oxybutynin base (50 mg) was introduced into a 25 ml volumetric flask and 15 ml distilled water were added. The mixture was sonicated for 15 minutes and completed to volume with water, then filtered on a 250 ml separating funnel. Oxybutynin base stock solution was prepared using the same procedure mentioned under "*Oxybutynin base stock standard solution"*. Further dilution of the stock sample solution was carried out and suitable aliquots were analyzed using the general procedures described for DDQ and *p*-chloranilic acid methods.

2.6. Stoichiometric relationship

Job's method of continuous variation was employed in order to establish the stoichiometry of the charge transfer complexes formed. Equimolar solutions of oxybutynin base and reagents (equivalent to 1.12×10^{-3} M and 2.38×10^{-3} M) were prepared in acetonitrile, in case of DDQ and *p*-chloranilic acid methods, respectively. Aliquots of the drug and reagent solutions in various complementary proportions (0.5 : 4.5, 1 : 4,. . ., 4 : 1, 4.5 : 0.5) were transferred into a series of 10 ml volumetric flasks, mixed and made up to the mark with acetonitrile. The absorbances of the resulting colored solutions were measured at 457 nm in DDQ method and at 520 nm in *p*-chloranilic acid method. Two curves relating the absorbance and the mole fraction of the drug were constructed and the molar ratios of the charge transfer complexes were obtained.

3. RESULTS AND DISCUSSION

Molecular interactions between electron donors and acceptors are generally associated with the formation of intensely colored charge transfer complexes and represent the basis for the determination of many drugs[15].

3.1. Absorption spectra

The reaction of oxybutynin as *n*-electron donor with DDQ as *π*-electron acceptor results in the formation of a red colored complex which exhibits absorption maximum at 457 nm, Fig. 2a. In addition, *p*-chloranilic acid acts as *π*-electron acceptor and the purple color formed exhibits an absorption maximum at 520 nm, Fig. 2b.

An attempt to use other *π*-electron acceptors such as TCNQ or *p*-chloranil for the determination of oxybutynin hydrochloride gave negative results.

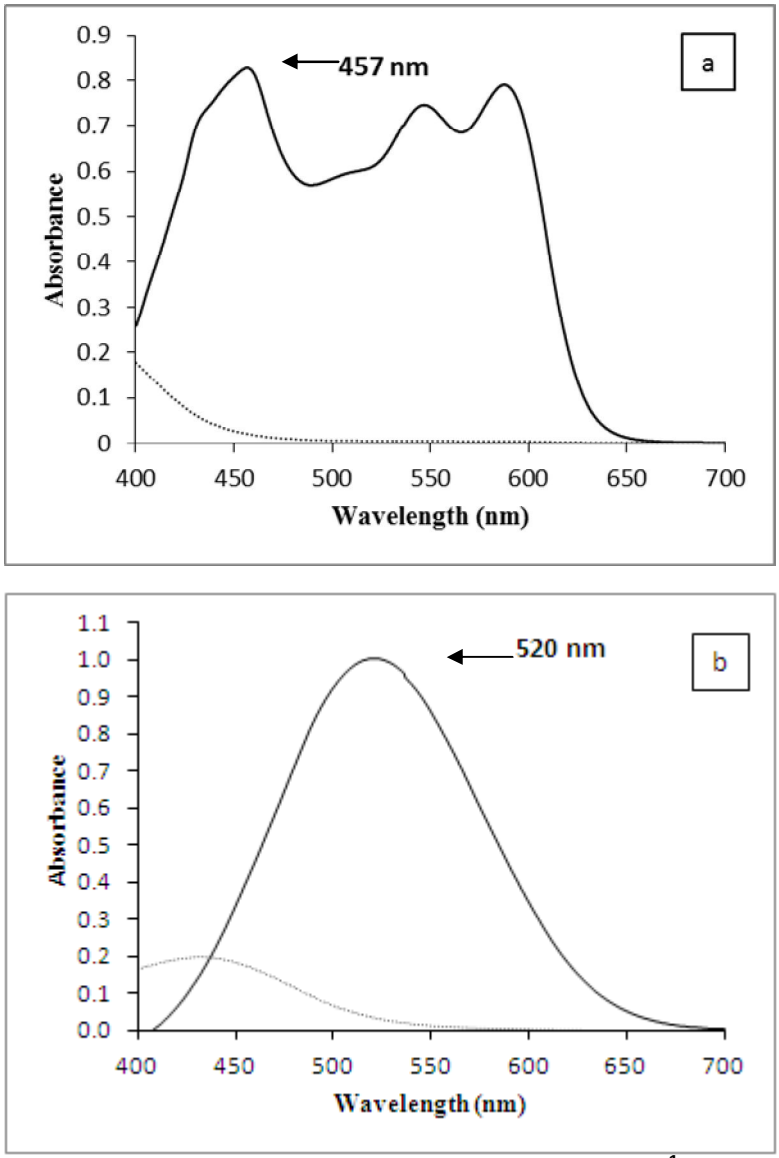


Fig. 2: Absorption spectra of oxybutynin (80 µg ml^{-1})/DDQ complex (___) and blank solution (……) in acetonitrile (a) and absorption spectra of oxybutynin (160 µg ml^{-1}) / *p*-chloranilic acid complex (___) and blank solution (…..) in acetonitrile (b)

3.2. Optimization of reaction conditions

The optimum conditions for the assay procedures have been established by studying the reactions as function of nature of the solvent, volume of reagent, temperature, the time needed for reaction completion and the stability of the color produced. Each factor was

studied in turn while keeping the others constant.

Effect of diluting solvent

The polarity of the solvent used in the reaction between π-acceptors with *n*-donors can influence the formation of charge transfer complexes. Therefore, investigations were carried out in order to choose the most favorable solvent for the formation of the colored products. The solvents studied were methanol[16], acetonitrile[17], acetone[18] and 1,4-dioxane[19].The solvent of choice was acetonitrile since it gave the highest absorption value with the greatest reproducibility in DDQ and *p*-chloranilic acid methods.

Effect of volume of reagent

The effect of volume of reagent on the intensity of the developed color at the selected wavelengths was ascertained by adding different volumes of DDQ solution (2 x 10^{-2} M) and *p*-chloranilic acid solution (2.38 x 10^{-3} M) to fixed concentrations of oxybutynin (80 µg ml^{-1}) and (160 µg ml^{-1}), for DDQ and *p*-chloranilic acid methods, respectively. It was found that 3 ml of DDQ and 4 ml of *p*-chloranilic acid solutions were sufficient for the production of maximum and reproducible color intensity, Fig. 3a and 4a. Further addition of reagents solutions caused no change in the absorbance.

Effect of temperature

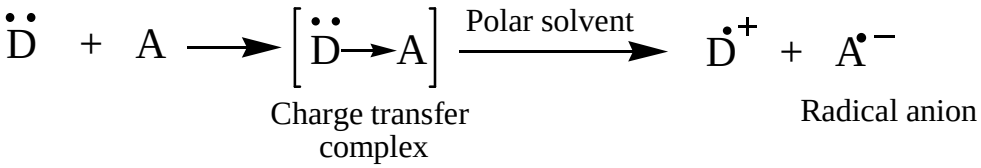
Different temperatures (20 - 55º C) were tested using a thermostatically controlled water bath in order to study the effect of temperature on the intensity of the produced color. It was found that both reactions are complete with the highest intensity at ambient temperature, Fig. 3b and 4b.

Effect of reaction time and stability of the color

The optimum reaction time was determined by following the color development upon the addition of DDQ and *p*-chloranilic acid solutions to the drug solution at ambient temperature. Complete color development was attained after 5 min and 10 min for DDQ and *p*-chloranilic acid methods, respectively, Fig. 3c and 4c.The absorbance of the developed colors remained stable for 1 hour, for both methods, Fig. 3d and 4d.

3.3. Stoichiometric relationship and reaction mechanism

The stoichiometry of the reaction between oxybutynin and each of DDQ and *p*-chloranilic acid was investigated by Job's method of continuous variation which revealed that the interaction occurred on equimolar basis, a donor to acceptor ratio of 1: 1, Fig. 5.This finding was anticipated by the presence of one *n*-donating center in the oxybutynin molecule, which is the basic tertiary amino group, Fig.6.The chemistry involved in the proposed methods is based on the reaction of the basic nitrogen of oxybutynin as *n*-donor (D)with the π-acceptor (A), DDQ and *p*-chloranilic acid to form charge transfer complexes of *n*-π type (DA). Due to the high ionizing power of the polar solvent, acetonitrile, dissociation of the complex was promoted and complete electron transfer from drug as an electron donor to acceptor moiety took place resulting in the formation of intensely colored radical anions of DDQ and *p*-chloranilic acid [20]. The reaction pathway was postulated to proceed as follows:



3.4. Method validation

The optimized spectrophotometric methods were validated by evaluating linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and selectivity in accordance with the ICH guideline Q2 (R1)[21].

Linearity

The linearity was investigated at six to seven concentration levels of the standard solution of oxybutynin base, each concentration was analyzed three times. The linearity was evaluated by linear regression analysis. The calibration curve was found to be linear over the concentration range of (20 - 80 µg ml^{-1}) and (30 - 160 µg ml^{-1}) for DDQ and *p*-chloranilic acid methods, respectively. The analytical data of the calibration curves, including standard deviations for the slope (S_b) and intercept (S_a), confidence limits of slope and intercept are summarized in Table 1.

Table 1: Analytical and validation parameters for the determination of oxybutynin with DDQ and *p*-Chloranilic acid

DDQ method		*p*-Chloranilic acid method	
Claimed taken (µgml^{-1})	Recovery[a]%	Claimed taken (µgml^{-1})	Recovery[a]%
25	99.83	44	100.79
35	100.19	60	99.31
40	100.03	80	99.88
55	98.94	120	99.39
65	100.43	140	99.03
75	99.80	150	99.51
Mean	99.87		99.65
± SD	0.512		0.623

Accuracy

The accuracy of the proposed methods was tested by analyzing triplicate samples of standard oxybutynin base solutions. The recovery percentages are stated in Table 2 and the results revealed the high accuracy of the proposed methods. Furthermore, the method accuracy was assessed as recovery obtained when spiking the sample solution with known concentrations of the intact drug (standard addition technique), Table 3.

Table 2: Determination of pure samples of oxybutynin by the proposed methods

Parameter	DDQ method	*p*-Chloranilic acid method
Wavelength	457 nm	520 nm
Range of linearity	20-80 µg ml^{-1}	30-160µg ml^{-1}
Regression equation	Y = 0.0093X+ 0.0619	Y = 0.0063X+0.0046
Regression coefficient (r^2)	0.9998	0.9999
S_b	6.61×10^{-5}	4.46×10^{-5}
S_a	0.0036	0.0045
LOD[a]	0.205	0.524
LOQ[a]	0.621	1.587
Confidence limit of the slope	$0.0093\pm 1.84\times10^{-4}$	$0.0063 \pm 1.15\times10^{-4}$
Confidence limit of the intercept	0.0619 ± 0.010	0.0046 ± 0.012
Standard error of the estimation	0.0035	0.0050
Intraday precision[b](RSD %)	0.218-0.373-0.225	0.398-0.245-0.220
Interday precision[c](RSD %)	0.385-0.615-0.172	0.609-0.280-0.230

[a]Limits of detection and quantification are determined via calculations[21]:
LOD= 3.3×SD/slope LOQ= 10×SD/slope
[b] The intraday (*n* = 3), average of three concentrations of oxybutynin (28, 44 and 72µg ml^{-1} for DDQ)and (40,100and 144µg ml^{-1} for *p*-chloranilic acid) repeated three times within the day.
[c] The interday (*n* = 3), average of three concentrations of oxybutynin (28, 44 and 72µg ml^{-1} for DDQ)and (40,100and 144µg ml^{-1} for *p*-chloranilicacid) repeated three times on three successive days.
[a]Average of three determinations

Table 3: Determination of oxybutynin in Uripan® tablets by the proposed methods and application of the standard addition technique

DDQ method				*p*-Chloranilic acid method			
Claimed (µg ml^{-1})	Recovery[a]% of Tablet	Pure added (µg ml^{-1})	Recovery[a]% of Added	Claimed (µg ml^{-1})	Recovery[a]% of Tablet	Pure added (µg ml^{-1})	Recovery[a] % of Added
20	99.52	20	100.54	40	100.16	40	99.61
		24	99.91			50	99.37
30	100.39	26	99.25	60	99.58	50	100.00
		30	99.28			60	99.47
40	100.57	32	99.80	80	99.88	70	99.32
		36	100.36			80	99.60
Mean	100.16		99.86		99.87		99.56
± SD	0.562		0.534		0.290		0.245

[a]Average of three determinations

Precision

The precision of the methods was checked by analyzing three different concentrations of oxybutynin base in triplicate during the same day (intraday precision). Interday precision study was determined by performing the same procedure on three consecutive days. The average recovery percentages were around 100% and the low percentage relative standard deviations (RSD %) indicated the good precision of the proposed methods, Table 1.

Selectivity

In order to evaluate the selectivity of the proposed methods for the analysis of oxybutynin in pharmaceutical formulation, the effect of the presence of tablet excipients namely lactose monohydrate, microcrystalline cellulose, corscarmellose sodium, magnesium stearate, colloidal silicon dioxide and citric acid anhydrous had to be taken in consideration. It was found that no interference was observed from any of these excipients with the proposed methods because of the insolubility of four of these excipients in water; the extracting solvent of oxybutynin hydrochloride from tablets. On the other hand, lactose monohydrate and citric acid are insoluble in chloroform; the extracting solvent of oxybutynin base. In addition, good percentage recoveries of tablets and the lower values of the RSD indicate high selectivity of the proposed methods.

Limit of detection and limit of quantification

According to the ICH recommendations, the parameters LOD and LOQ were determined on the basis of standard deviation of the response and slope of the regression equation, Table 1. The DDQ method was superior to *p*-chloranilic acid method due to lower detection limit.

Statistics

A statistical comparison was performed between the proposed methods and the reference method[14]. By using student's t-test and variance ratio F-test, it was found that the values of calculated t and F are less than the tabulated ones which reveals that there is no significant difference between the proposed and the reported methods with respect to accuracy and precision, Table 4.

Table 4: Statistical comparison of the results obtained by applying the proposed methods and the reference method for the analysis of oxybutynin in pure sample

Item	DDQ method	*p*-Chloranilic acid method	Reference method**
Mean± SD	99.87± 0.512	99.65±0.623	100.05±0.703
n	6	6	6
Variance	0.262	0.388	0.494
SE	0.209	0.254	0.287
Student's t test	0.507 (2.228*)	1.044 (2.228*)	
F-ratio	1.885 (5.050*)	1.273(5.050*)	

* The values in the parenthesis are the corresponding values of t and F at (*p*=0.05)

** Reference method[14]

4. CONCLUSION

The present investigation is based on well-characterized charge transfer complexation reaction. It exploited the aliphatic tertiary amino group in oxybutynin molecule and used DDQ and *p*-chloranilic acid which represents common and simple analytical reagents that can be afforded by any ordinary analytical laboratory. The developed spectrophotometric methods are proved to be simple, sensitive and selective and do not involve any complex steps and gives accurate and precise results. Moreover, the analysis was carried out at room temperature using inexpensive equipments compared to the reported HPLC and LC/MS methods. Satisfactory results of the determination of oxybutynin in pharmaceutical preparation reveal that there is no interference of the usual excipients. Therefore, it can be concluded that the proposed methods can be applied for the routine analysis of oxybutynin in quality control laboratories.

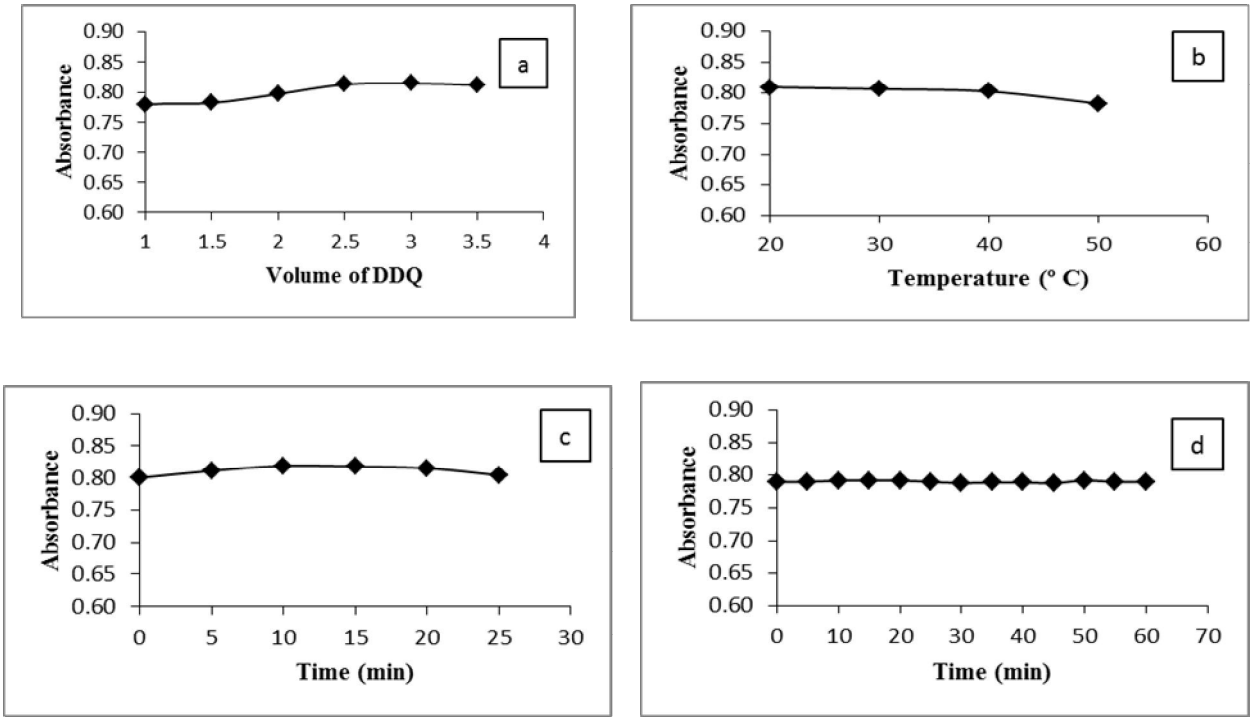


Fig. 3: Effect of the volume of DDQ (a), temperature (b) and reaction time (c) on the reaction of oxybutynin (80 µg ml^{-1}) with DDQ (2x10^{-2} M) and effect of time on the stability of the formed charge transfer complex (d).

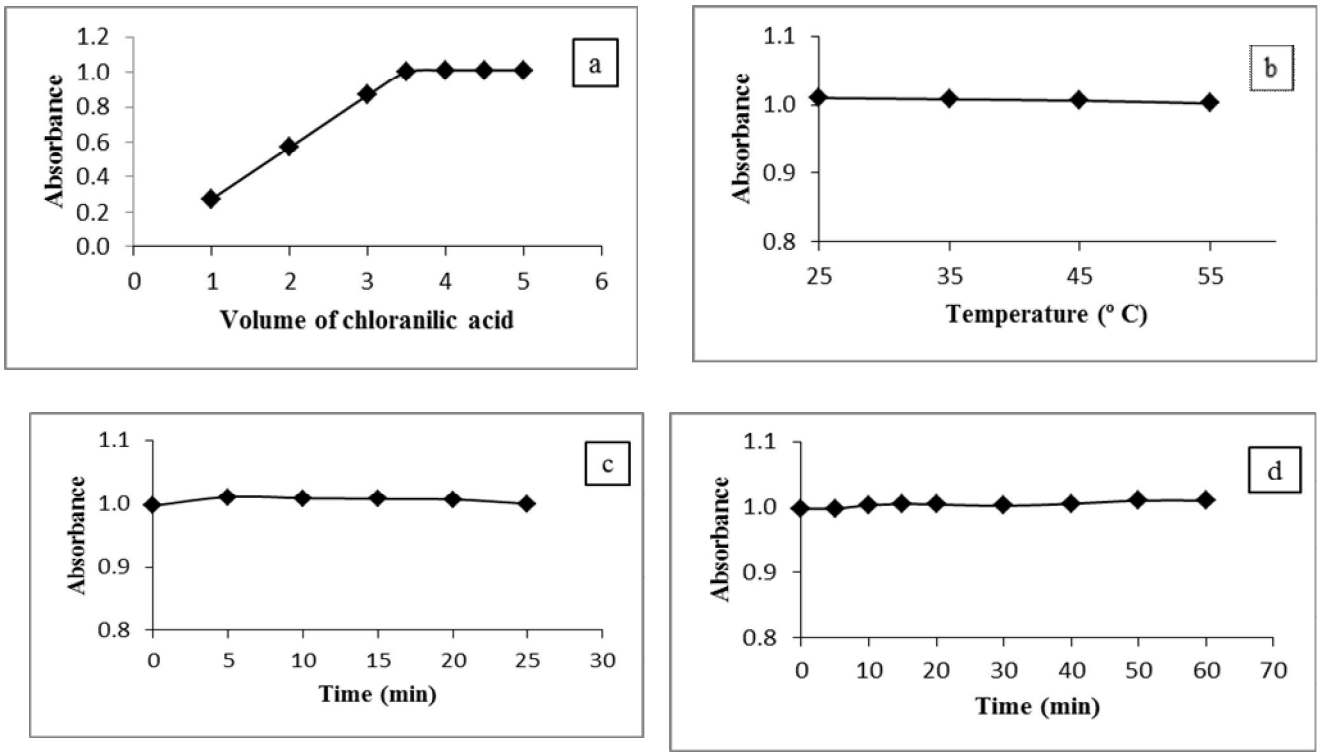


Fig. 4: Effect of the volume of *p*-chloranilic acid (a), temperature (b) and reaction time (c) on the reaction of oxybutynin (160 µg ml^{-1}) with *p*-chloranilic acid (2.38x10^{-3} M) and effect of time on the stability of the formed charge transfer complex (d).

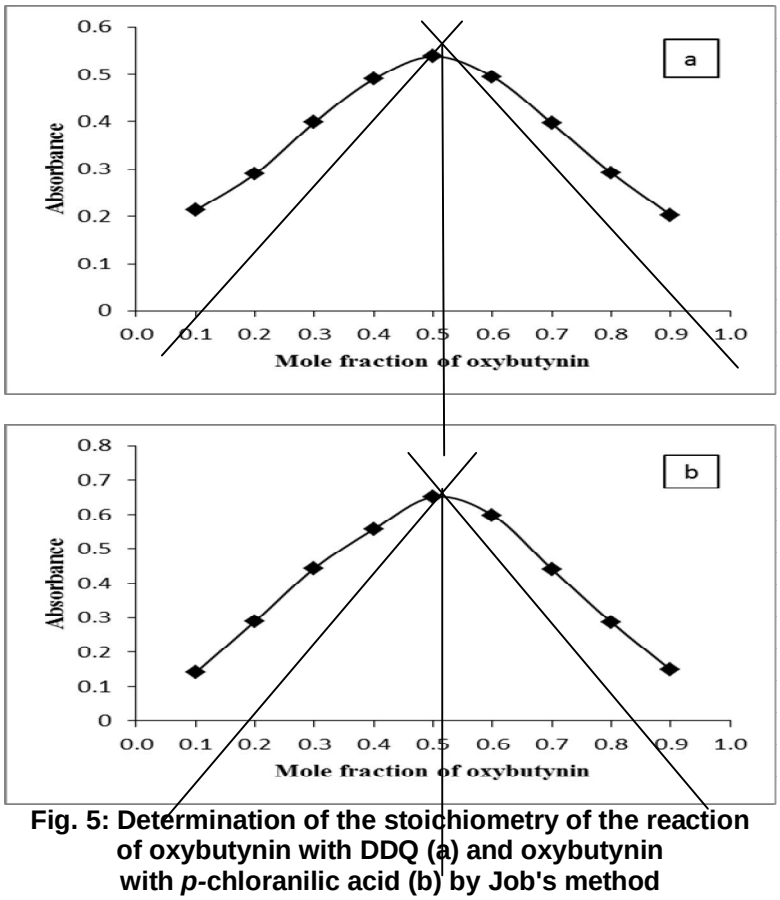


Fig. 5: Determination of the stoichiometry of the reaction of oxybutynin with DDQ (a) and oxybutynin with *p*-chloranilic acid (b) by Job's method

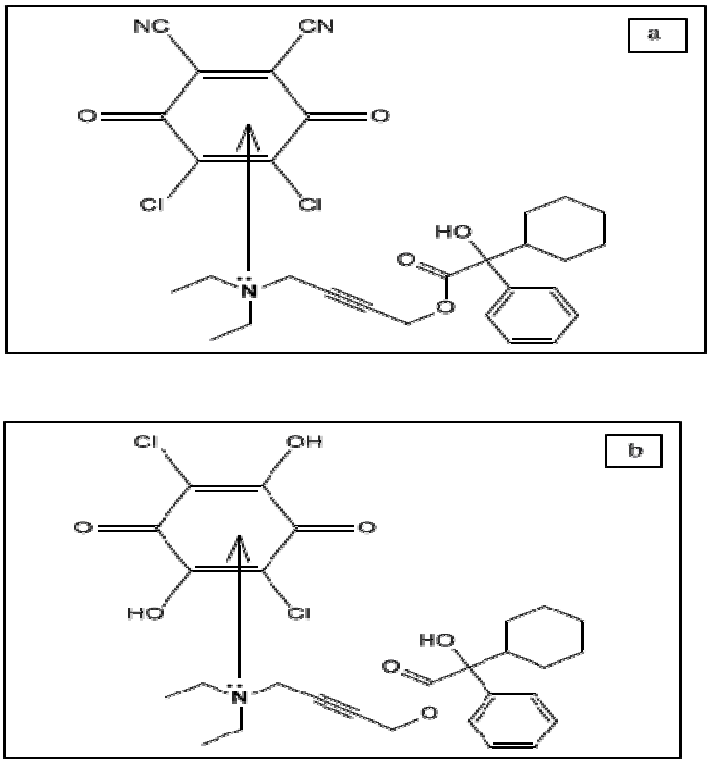


Fig. 6: The suggested structures of oxybutynin / DDQ (a) and oxybutynin / *p*-chloranilic acid (b) charge transfer complexes

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