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2,6-Dichloroquinone chlorimide and 7,7,8,8-tetracyanoquinodimethane reagents for the spectrophotometric determination of salbutamol in pure and dosage forms

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Abstract

A simple, rapid and sensitive spectrophotometric method for the determination of sulbutamol in pure form and in different pharmaceutical preparations has been developed. The charge transfer (CT) reaction between salbutamol as electron donor and 2,6-dichloroquinone chlorimide (DCQ) and 7,7,8,8-tetracyanoquinodimethane (TCNQ) as a π-electron acceptor have been spectrophotometrically studied. The optimum experimental conditions for these CT reactions have been studied carefully. Beer's law is obeyed over the concentration range of 1.0–30.0 μg ml^{-1} and 2.0–20.0 μg ml^{-1} for salbutamol using DCQ and TCNQ, respectively. For more accurate results, Ringbom optimum concentration range is calculated and found to be 10.0 to 30.0 and 8.0 to 20 μg ml^{-1} for salbutamol using DCQ and TCNQ, respectively. The Sandell sensitivity is found to be 0.011 and 0.010 g cm^{-2} for salbutamol using DCQ and TCNQ, respectively, which indicate the high sensitivity of the proposed methods. Relative standard deviations (R.S.D.) of 0.27 to 0.68% and 0.20 to 1.40% ($n = 5$) were obtained for five replicates of salbutamol using DCQ and TCNQ, respectively. The results obtained by the two reagents are comparable with those obtained by British pharmacopoeia assay for the determination of salbutamol in raw materials and in pharmaceutical preparations.

Keywords: Spectrophotometric; Salbutamol; 2,6-Dichloroquinone chlorimide (DCQ); 7,7,8,8-Tetracyanoquinodimethane (TCNQ); Pharmaceutical preparations

1. Introduction

The charge transfer (CT) reactions had been widely studied spectrophotometrically in the determination of drugs that are easy to be determined based on CT complex formation with some electron acceptors. 2,6-Dichloroquinone chlorim-

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ide (DCQ) and 7,7,8,8-Tetracyanoquinodimethane (TCNQ) are strong electron acceptors and applied in the determination of several electron donors drugs and the review of literature in the last decade had been mainly concentrated on the CT-complexes spectral studies [1–6]. Salbutamol is a direct-acting sympathomimetic agent with a relatively selective action on β2-adrenoacceptors. Its clinical uses are in the management of a reversible airways observation such as that occurs in asthma and delaying premature labour [7]. Normal and derivative spectrophotometric methods [8,9], flow injection analysis [10], electrophoresis [11], thin layer chromatography [12,13], gas and liquid chromatographic methods [14,15] were used as quantitative tools for the determination of salbutamol.

The present research aims chiefly to study the reaction of both DCQ and TCNQ reagents (electron acceptors) as first time with salbutamol drug (electron donor) and to use these reagents in spectrophotometric determination of the given drug in pure form and in some of its pharmaceutical preparations.

2. Experimental

2.1. Instruments

Shimadzu Model 160A UV–Visible double beam spectrophotometer with a 1.0 cm quartz cells was used. An Orion Research Model 601A/Digital Ion Analyser was used for checking the pH of the universal buffer solutions.

2.2. Reagents

All chemicals and solvents used were of analytical or pharmaceutical grade. All solutions were prepared in doubly distilled water. Pure drug standard samples were supplied by Galaxo Welcome pharmaceutical company (Egypt). Dosage forms containing salbutamol were purchased from local market companies. DCQ and TCNQ were supplied by Aldrich Company, USA.

Fresh solutions of DCQ (0.1% w/v) in isopropanol and of TCNQ solution (0.02% w/v) in acetonitrile were freshly prepared. Universal buffer solutions of different pH values 3.0 to 12.0 were also prepared. Standard Salbutamol solutions containing 2.0 mg ml^{-1} in water and 0.2 mg ml^{-1} in acetonitrile were freshly prepared for studying reactions with DCQ and TCNQ reagents, respectively.

2.3. Procedure

2.3.1. bulk sample

Aliquots containing salbutamol in the working concentration range of 10.0–300 and 20–200 μg ml^{-1} for DCQ and TCNQ reagents, respectively, were transferred into 10.0 ml measuring flask. About 1.0 ml of buffer solution of pH 9.0 was added in case of DCQ only, then 1.0 ml of DCQ or 2.0 ml of TCNQ solution was added. The solution was completed to the mark with distilled water in case of DCQ and with acetonitrile in case of TCNQ. The solution was left to stand for few minutes at room temperature before the absorbance was measured at $\lambda = 602$ and 842 nm for DCQ and TCNQ, respectively, against a blank solution prepared in the same manner without drugs. The drug concentration was calculated from the standard calibration graph prepared under the same identical conditions.

2.3.2. Pharmaceutical dosage forms (tablets)

2.3.2.1. Using DCQ reagent. Weigh and thoroughly grind 20 tablets. Extract an accurately weighed portion of the obtained powder equivalent to 50.0 mg of salbutamol with 25.0 ml distilled water. Shake for about 15.0 min, filter the mixture into 100 ml measuring flask and wash the residues several times and dilute to the mark with water. The analysis was continued as described above and the nominal constant was calculated from the corresponding calibration graph or regression equation.

2.3.2.2. Using TCNQ reagent. An accurately weighed amount of the finely powdered 20 tablets equivalent to about 10.0 mg of salbutamol was transferred into a 50.0 ml beaker. This beaker was half-filled with DMF and heated in a boiling

water-bath for 20.0 min [8]. The solution was cooled, transferred into 50.0 ml volumetric flask and diluted to volume with the same solvent. The solution was filtered and the first few milliliters of the filtrate were discarded. Different aliquots from the filtrate were measured by pipette and the method was continued as mentioned above [8].

3. Result and discussion

3.1. Absorption spectra

The absorption spectra of the reaction product between salbutamol (containing phenolic group) and DCQ reagent in alkaline medium (pH 9.0) is shown in Fig. 1. It shows a characteristic maxima at $\lambda_{max} = 602$ nm [16]. The absorption spectra of the colour product-CT complex in the universal buffer solution of varying pH values (3.0–12.0) were recorded in order to select the optimum pH. This also gives us an idea about the possible species that can exist in such media. The spectral measurements in the visible region show no blue or red effect of pH variation on λ_{max} at 602 nm. Fig. 2 shows an increase in the absorbance with the increase of pH at the specific wavelength till pH 9.0 (maximum absorbance). At pH more than 9.0, has a stable value (Fig. 2B) by increasing of pH. So the optimum pH value of this reaction is taken between 10 and 12 inspite of the maximum absorbance at pH 9, because little lower pH may cause high error.

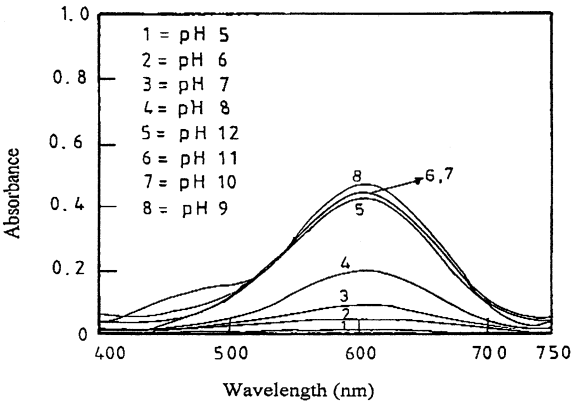


Fig. 2. Effect of pH on the reaction of salbutamol with DCQ.

TCNQ in acetonitrile reacts with the amino group of salbutamol and give an intense bluish–green CT complex which absorb maximally at $\lambda = 842$ nm (Fig. 3). The CT complex was formed by the interaction of the investigated drug base as n-electron donors and TCNQ as π-acceptor.

The spectrophotometric properties of the coloured CT complexes as well as the different parameters affecting the colour development between salbutamol and both reagents were extensively studied to determine the optimal conditions for the assay procedure. The reaction was studied as a function of the volume of the reagent, nature of solvent, reaction time, temperature but it does not stability indicating.

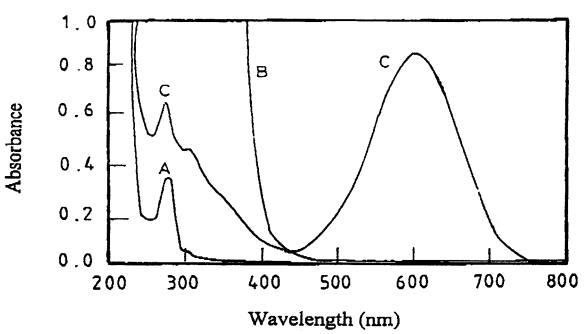


Fig. 1. Absorption spectra of (A) salbutamol solution, (B) DCQ reagent and (C) salbutamol-DCQ product.

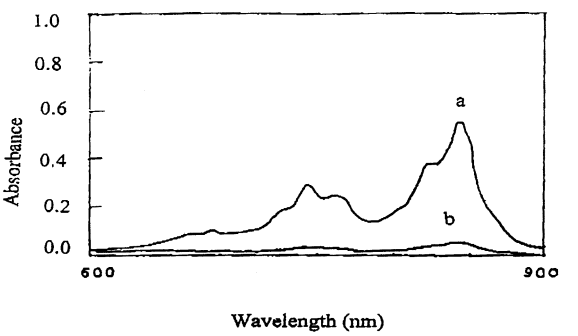


Fig. 3. Absorption spectra of (A) salbutamol TCNQ complex in acetonitrile and (B) TCNQ in acetonitrile.

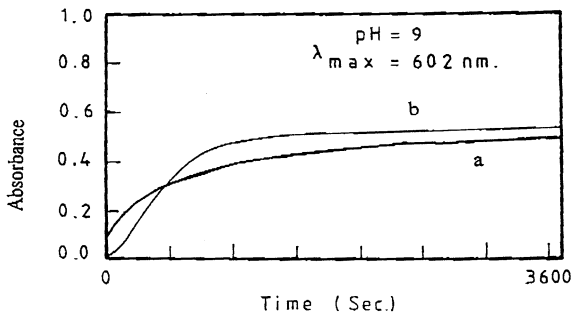


Fig. 4. Effects of time on the spectra of the reaction products: (a) salbutamol-DCQ and (b) salbutamol-TCNQ.

3.2. Effect of reaction time

The reaction time is determined by following the colour development at different time intervals at room temperature. It is found that, maximum absorbance is attained after 15 min. The colour remains constant for more than 1 day (Fig. 4). This indicates the colour stability of reaction product, which permits the use of the procedure in microdetermination of the drug in acceptable duration.

3.3. Effect of DCQ and TCNQ concentration

For DCQ reagent, it was found that, maximum absorbance was attained using 1.0 ml of 0.1% DCQ solution and 1.0 ml of pH 9.0 of the universal buffer solution. The colour developed after 30.0 min and still constant for more than 1 day (Fig. 4). For TCNQ ragent, the maximum absorbance is attained using 1.0 ml of 0.02% TCNQ solution.

3.4. Effect of solvent

To select the solvent that would give the highest absorbance, different solvents were tested like methanol, ethanol and different mixtures of ethanol:acetonitrile. It was found that, the response between absorbance and concentration was non linear. Acetonitrile was considered as an ideal solvent because it offered an excellent solvating power for TCNQ reagent to give high absorbance [17,18].

3.5. Calibration and precision

Typical calibration data obtained for salbutamol drug obtained from linear regression analysis of absorbance readings versus concentration of the drug (μg ml^{-1}) were made. The slope, intercept, Sandell sensitivity (S), apparent absorptivities and correlation coefficient were listed in Table 1. Beer's law limits are 1.0–30 and 2.0–20 μg ml^{-1} for salbutamol using DCQ and TCNQ reagents, respectively. Moreover, Ringbom optimum concentration ranges can be calculated which give more accurate results at 10–30 and 8.0–20.0 μg ml^{-1} salbutamol using DCQ and TCNQ, respectively. The apparent molar absorptivities of the resulting colour products CT complexes were 2.25×10^4 and 2.8×10^4 l mol^{-1} cm^{-1} for salbutamol using DCQ and TCNQ reagents, respectively.

Five replicate measurements are performed at two different concentrations (10 and 15 μg ml^{-1}) and the following relative standard deviations (R.S.D.) of 0.27–0.68 and 0.20–1.4 for DCQ and

Table 1
Analytical and spectral characteristics of the coloured products, precision and accuracy

Parameters	Reading	
	DCQ method	TCNQ method
λ_{max} (nm)	602	842
pH	9.0	–
Molar absorptivity (l mol^{-1} cm^{-1})	2.25×10^4	2.80×10^4
Sandell sensitivity (μg cm^{-2})	0.011	0.010
Ringbom sensitivity (μg ml^{-1})	10.0–30.0	8.0–20.0
Beer's law limit (μg ml^{-1})	to 30.0	to 20.0
Range of error (%)	0.174 to 0.82	0.40 to 1.60
Relative standard deviation (%)	0.27 to 0.68	0.2 to 1.4
Regression equation[a]		
Slope (b)	0.973	1.008
Intercept (a)	0.546	0.052
Coefficient of determination (r^2)	0.996	0.996

[a] $A = a + bC$, where C is the concentration in μg ml^{-1}.

Table 2
Assay of salbutamol in bulk and dosage forms by the proposed and official methods

Sample	Concentration taken (μg ml^{-1})	Proposed methods, found ± S.D.%[d]		Official method	*t*-test		*F*-test	
		DCQ (μg ml^{-1} ± S.D.%[d])	TCNQ (μg ml^{-1} ± S.D.%)	Found ± S.D.%[d] (μg ml^{-1} ± S.D.%[d])	DCQ	TCNQ	DCQ	TCNQ
Salbutamol (bulk)	10.0	9.91 ± 0.6	10.11 ± 0.30	9.99 ± 0.68	2.57	2.60	5.58	6.4
D_1[a]	10.0	9.87 ± 0.90	9.965 ± 0.52	9.97 ± 0.25	2.35	2.40	3.76	3.00
D_2[b]	10.0	9.904 ± 0.93	9.942 ± 0.65	9.97 ± 0.24	1.60	2.30	3.88	2.70
D_3[c]	10.0	9.96 ± 1.20	9.91 ± 0.42	9.99 ± 0.27	2.48	2.48	4.40	2.00

Where tabulated value of *t*-test under confidence limit 95% = 2.776 tabulated value of *F*-test under confidence limit 95% = 6.39. Degree of freedom ($v_1 = 4$, $v_2 = 4$), number of replicates ($n_1 = 5$, $n_2 = 5$).

[a] D_1, Salbolin tablet (2.0 mg per tablet), The Arab Drug Company, Egypt.

[b] D_2, Bronchovent tablet (2.0 mg per tablet), MISR Co for Pharm. Ind., Egypt.

[c] D_3, Salbovent tablet (2.0 mg per tablet), The Alex Co, Egypt.

[d] Number of replicates, $n = 5$.

Table 3
Between-day measurements of CT reactions between salbutamol and TCNQ or DCQ

Compound	*W* taken (μg ml^{-1})	*W* found (μg ml^{-1})	Percentage recovery (%)	S.D.	R.S.D. (%)
TCNQ method	6	5.95	99.17	0.08	1.34
	10	9.95	99.5	0.09	0.90
	12	12.15	101.25	0.13	1.07
DCQ method	5	4.94	98.80	0.06	1.21
	10	9.95	99.50	0.07	0.70
	15	14.95	99.33	0.09	0.60

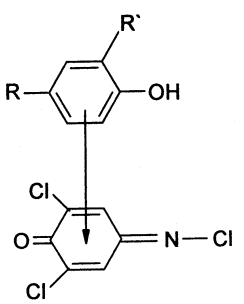
TCNQ, respectively, are obtained. It indicates the high accuracy and precision of the proposed method in the determination of salbutamol. The performance of the proposed method was assessed by comparison with the British pharmacopoeia [19]. Comparison through *F*- and *t*- tests [20] showed the equivalency of this method (Table 2).

3.6. Stoichiometry

A further study on the CT complex reaction of salbutamol with DCQ and TCNQ, the stoichiometry of the reaction mixture was determined by using continuos variation method. The results show a 1:1 salbutamol:reagents (DCQ or TCNQ) CT complexes.

3.7. Mechanism of reaction between DCQ and TCNQ with salbutamol

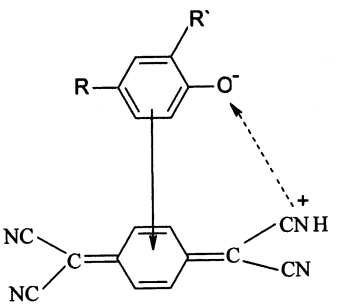
DCQ is an electron acceptor and the benzene ring in salbutamol molecule is the electron rich group, so a π–π* CT complex is formed [5]:



(R = CH(OH)—CH_2—NH—Bu` , R` = CH_2OH)

DCQ-salbutamol

The CT complex formed between salbutamol and TCNQ reagent takes place through the migration of H^+ ion to one of the four cyano groups in TCNQ reagent to form positive ion which associate with the phenolate anion to form ion pairs as follows [18]. Also, π–π* CT complex is formed via the benzene ring (electron rich group) of the salbutamol drug and TCNQ reagent (electron acceptor) [5,18].



(R = CH(OH)—CH_2—NH—Bu` , R` = CH_2OH)

TCNQ-salbutamol

3.8. Interference

Interference were those which could cause analytical problems. It was found that, the proposed methods could be applied to determine salbutamol in different preparations without any analytical problems. On the other hand, tablet fillers such as starch, lactose, glucose and stearic acid did not interfere in the proposed method.

3.9. Application

The two proposed methods are applied to the determination of salbutamol in dosage forms

(commercial products randomly collected from local pharmacies). Table 2 lists the results obtained by the proposed and British pharmacopoeia [19] based on electrometric titration with 0.1 N perchloric acid. The results indicate good agreement with the official method. The proposed colourimetric methods can be recommended for routine analysis in the majority of drug quality control laboratories. Another favourable characteristic of the method is that the absorbances of the coloured products formed are stable for at least 24.0 h.

On comparison of the results obtained by the proposed methods with the pharmaceutical method [19] using the t-test for the accuracy and F-test for the precision assessment [20], the calculated values did not exceed the corresponding theoretical values (tabulated value of t- and F-test under confidence limit 95% = 2.776 and 6.39, respectively) indicating insignificant differences between the results and also refer to the robustness of the proposed procedure. The proposed method is more accurate and of high robustness, with high recoveries amounting to 98.70 to 99.60 ± 0.60 to 1.20% for DCQ and 99.10 to 101.1 ± 0.30 to 0.65% for TCNQ compared with 99.68 to $99.90 \pm$ 0.24 to 0.68% using the British pharmacopoeia. The day by day availability of the proposed method is given in Table 3. It shows the values of the between-day R.S.D. for different concentrations of the drugs, obtained from experiments carried out over a period of four days. It gives a S.D. of 0.06 to 0.13 and 0.06 to 0.09 for TCQ and DCQ methods and RSD of 0.08 to 1.21 and 0.09 to 1.34 for TCQ and DCQ methods, respectively, referring to the high accuracy and precision and robustness of the applied procedures.

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