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Utilization of charge transfer complex formation for the spectrophotometric determination of piroxicam and tenoxicam

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ABSTRACT

The molecular interaction between piroxicam and tenoxicam as electron donor and each of 7,7,8,8-tetracyanoquinodimethane (TCNQ), tetracyanoethylene (TCNE) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) as electron acceptor have been investigated spectrophotometrically. The coloured products are measured at 844, 393 and 459 nm for TCNQ; TCNE and DDQ respectively. Optimization of the different variables affecting the reaction is described. The TCNQ, TCNE and DDQ-based color systems were stable for 3 hrs in non-aqueous media and obeyed Beer's law over a wide range of concentration. The linear working ranges, apparent molar absorptivities, Sandell's sensitivity indexes, detection and quantification limits were calculated for all systems. Job's plot of the absorbance versus the mole fraction of the drug indicated the formation of a 1:1 adducts. Application of the procedure to the analysis of various pharmaceutical samples gave reproducible and accurate results. Further, the validity of the procedure was confirmed by applying the standard addition technique.

KEYWORDS

Charge transfer complexes;
Spectrophotometry;
Piroxicam;
Tenoxicam;
TCNQ;
TCNE;
DDQ.

INTRODUCTION

Piroxicam (PX), 4-hydroxy-2-methyl-N-(2-pyridinyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide, is a non-steroidal anti-inflammatory agent which is widely used in the treatment of rheumatic diseases[1]. The employment of several analytical methods (voltammetry, polarography, ion selective electrode, spectrofluormetry and chromatography) for the determination of PX in pharmaceutical samples and biological fluids has been reported[2-10]. On the other hand, the use of spectrophotometry for the quantification of PX was reported[11-13]. Since spectrophotometry has the advantage of both sensitivity and simplicity, it has found extensive use in the determination of inorganic, organic and bioactive materials[14].

Tenoxicam (TX) [4-hydroxy-2-methyl-N-2-pyridyl-2H-thieno-(2,3-e)-1,2-thiazine-3-carboxamide-1,1-dioxide] is a relatively new non-steroidal drug which has anti-inflammatory, analgetic and antipyretic effects. It is a derivative of oxicam with a thiophene ring replacing the benzene ring in piroxicam. Tenoxicam inhibits cyclooxygenase which catalyses the formation of cyclic endoperoxides[15]. Several methods for the determination of tenoxicam have been re-

ported in literature, such as liquid chromatography[16], high performance liquid chromatography[17, 18], spectrophotometric[14, 19-22] as well as the electroanalytical methods[3, 23].

The British pharmacopoeia describes a chromatographic and non aqueous titration methods for the determination of piroxicam and tenoxicam, respectively[24].

The purpose of the present work was to develop an alternative simple method which would require inexpensive equipment. The method could also be used to confirm HPLC results because of its simplicity. The method is based on charge transfer complex formation between the drugs and TCNQ, TCNE and DDQ as acceptors.

EXPERIMENTAL

Apparatus

Electronic absorption spectra were recorded on a Shimadzu 1601 UV-Vis. Spectrophotometer.

Materials

Piroxicam and tenoxicam were purchased from Sigma (St. Louis, MO, USA). However, their dosage forms (fledene tablets, fledene capsules, fledene suppositories, epicotil tablets and epicotil suppositories) were purchased from the local market.

100 μg ml^{-1} standard stock solutions of piroxicam and tenoxicam were prepared by dissolving 25 mg of the drug in 250 ml methanol or acetonitrile. $5x10^{-3}$ M (PX) and (TX) solutions were prepared by dissolving an appropriate weight in 100 ml of methanol or acetonitrile in the same manner.

Reagents

All reagents and solvents used were of analytical reagent grade. 7,7,8,8-tetracyanoquinodimethane (TCNQ), Aldrich, Milwaukee, WI, USA and tetracyanoethylene (TCNE), Nacalai Tesque, Kyoto, Japan. $5x10^{-3}$ M solution were prepared in acetonitrile, the solutions were stable for at least one week at 4.0 ^{0}C. 2, 3-dichloro-5,6-dicyano-p-benzoquinone (DDQ), Merck, Darmstadt, Germany. Stock solution of $5x10^{-3}$ M was prepared by dissolving an accurate weight in methanol in a 100 ml calibrated flask.

General procedures

Method using TCNQ and TCNE

Aliquot volumes containing 75-575 μg of PX or 75-550 μg TX were placed in 25 ml grade A calibrated flask, followed by 5.0 ml of $5x10^{-3}$ of TCNQ and was heated in a water bath at 70±1^{0}C for 10 min. The reacting mixture was cooled, completed to volume with acetonitrile and the absorbance was measured at 844 nm, against a reagent blank prepared in the same way.

Aliquot volumes containing 75-525 μg PX or 75-500 μg TX were placed in 25 ml grade A calibrated flask, followed by 5.0 ml of $5x10^{-3}$ of TCNE and was heated in a water bath at 70±1^{0}C for 10 min. The reacting mixture was cooled, completed to volume with acetonitrile and the absorbance was measured at 393 nm against a reagent blank prepared in the same way.

Method using DDQ

A standard solution containing 37.5-650 μg PX or 37.5-600 μg TX was transferred to 25 ml calibrated flask, followed by 6 ml of $5x10^{-3}$ M DDQ solution and heated in a water bath at 70±1^{0}C for 10 min. The reacting solution was cooled, diluted to volume with methanol and the absorbance was measured at 459 nm, against a reagent blank prepared in the same manner.

Determination of Molar ratio

Job's method of continuous variation was employed, $5x10^{-3}$ M standard solution of drug and reagents were used. A series of solutions was prepared in which the total volume of the drug and reagent was constant (5 ml). The drug and reagents were mixed in various proportions and then diluted in 25 ml calibrated flasks with the optimum solvent. The absorbance was measured after treating each reagent at the best time and temperature against a reagent blank under the same conditions.

Procedure to tablets and capsules forms

The contents of twenty capsules or finely ground tablets were weighed and mixed. An amount of the tablet powder or capsule powder equivalent to 100 mg of PX or TX was weighed, dissolved in acetonitrile or methanol and any remaining residue was removed by filtration. The clear solution was diluted with the solvent

used in a 100 ml calibrated flask. The drug content of this solution was obtained by applying the general procedure using the similarly prepared calibration graph.

Procedure to suppositories form

At least ten suppositories were weighed, cut into small pieces and transferred to a small porcelain dish. They were melted by stirring in a water bath to homogenize and cooled. The weighed portions equivalent to 25 mg PX or TX were transferred into a beaker, melted and dissolved into acetonitrile or methanol, by stirring using a magnetic stirrer at 60±1 ^{0}C. The solution was cooled, filtered, and diluted with the solvent used in a 250 ml calibrated flask and analyzed as described above under the general procedures using the similarly prepared calibration graph.

RESULTS AND DISCUSSION

Substituted quinones (DDQ and TCNQ) and TCNE were reported to possess the property of accepting electrons from electron donors[25-27]. This property results in a complete electron transfer from donor to acceptor moieties.

The reaction of DDQ reagent with PX or TX results in the formation of an intense orange-red coloured product that exhibits an absorption maximum at 459 nm. This spectrum is similar to that obtained by reduction of DDQ with iodine[28].

In acetonitrile, a solution of PX or TX and reagent TCNQ yields an intense blue colour, producing a characteristic long wavelength absorption band, frequently with numerous vibrational maxima in the electronic spectrum. The predominant chromogen with TCNQ is the blue radical anion which was probably formed by the dissociation of an original donor-acceptor (DA) complex with PX or TX:

$$D + A \underset{\text{DA Complex}}{\overset{\text{Polar Solvent}}{\rightleftharpoons}} D\text{-}A \rightleftharpoons \underset{\text{Radical Ions}}{D^- + A^+}$$

The studied drugs have high electron densities and act as powerful electron donors. In polar solvent, PX and TX exhibit maxima in the UV region at 250-320 nm. Upon addition of TCNQ, TCNE or DDQ a pronounced bathochromic shift is observed. This change in the spectra may be attributed to the formation of the charge transfer complex.

Investigations were carried out to establish the most favorable conditions that give highly intense colors and to achieve maximum colour development in the quantitative determination of PX and TX. The quantitative parameters for complex formation of PX and TX using TCNQ, TCNE and DDQ are listed in TABLE 1. The influence of various reaction conditions on the color systems was investigated.

Effect of solvent

The effect of solvent on the formation of the charge transfer complex was studied using methanol, ethanol, propanol, acetone, dioxane, dimethylformamide and acetonitrile. No reaction occurred in dioxane or propanol. Acetonitrile was preferred to acetone because of the higher molar absorptivity of the complexes of TCNQ and TCNE. On the other hand, acetone needs a longer time to achieve maximum colour development. For DDQ complexes, methanol was found to be an ideal solvent for the colour reaction and gives maximum absorbance at their absorption bands.

Effect of reagent concentration

The results for variation of reagent concentration indicated that 3.5 ml of either TCNQ or TCNE are suitable whereas using DDQ 5 ml is sufficient for complete colour intensity. Figure 1 shows the effect of reagent concentration on the PX-based color systems. The higher concentrations of the reagent may, on the other hand, be useful for rapidly reaching equilibrium and complete colour development. This minimizes the

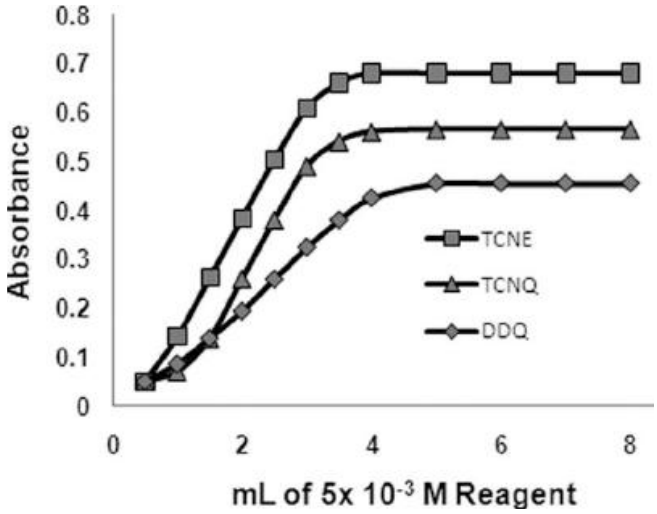

Figure 1 : Effect of reagent concentration on the color systems containing 15 μ g ml^{-1} PX.

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TABLE 1 : Quantitative parameters for complexation of PX and TX using TCNQ, TCNE and DDQ

Parameters	TCNQ		TCNE		DDQ	
	PX	TX	PX	TX	PX	TX
Solvent	Acet	Acet	Acet	Acet	MeOH	MeOH
Time (min)	10	10	10	10	10	10
Temperature °C	70	70	70	70	70	70
Reagent conc.X10^3	1.0	1.0	1.0	1.0	1.2	1.2
λ_{max} (nm)	844	844	393	393	459	460
Beer's conc.range (μg ml^{-1})	3-23	3-22	3-21	3-20	1.5-26	1.5-24
Ringbom conc. range (μg ml^{-1})	5-21	5-20	5-19	4-18	3-24	3-22
Detection limits (μg ml^{-1}), 3σ	0.87	0.95	0.92	0.90	0.46	0.50
Quantification limits (μg ml^{-1}), 10σ	2.9	3.1	3.0	3.0	1.43	1.6
Molar absorptivity X10^4 (L $mol^{-1}cm^{-1}$)	1.27	1.38	1.53	1.43	1.03	1.33
Sandell sensitivity (μg cm^{-2})	26.14	24.44	21.53	23.53	32.10	25.26
Regression equation*						
Intercept (a)	-0.003	0.007	0.009	0.005	-0.011	0.008
Slope (b) 10^{-2}	3.83	4.09	4.62	4.25	3.12	3.96
Correlation coefficient (r)	0.9990	0.9988	0.9996	0.9994	0.9998	0.9992

***A = a+bc, where c: is the concentration in μg ml^{-1}; Acet = Acetonitrile, MeOH = Methanol**

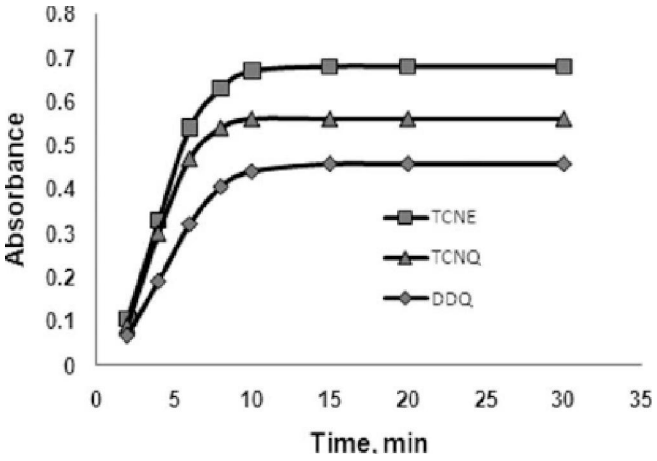

Figure 2 : Effect of time on the color systems containing 15 μ g ml^{-1} PX at 70 °C.

time required to attain the maximum absorbance at the corresponding wavelength of the charge transfer complex. Hence, using 5 ml of TCNQ or TCNE and 6 ml of 5x10^{-3} M DDQ must be used due to highly concordant results.

Effect of temperature and heating time

The optimum reaction time was determined by following the colour intensity at ambient temperature (25±2 °C). Complete color development at 25 °C was attained after 60, 75 and 45 min using TCNQ, TCNE and DDQ, respectively. On raising the temperature to 70 °C, complete color development was obtained after 10 min only. Figure 2 shows the effect of heating time on the PX-based color systems. The color remained stable for 3 hrs for all reagent complexes. Latter the absorbance gradually decreased with blue shift in λ_{max} until the band disappeared completely.

Stoichiometric relationship

Job's continuous variation graph for the reaction between PX or TX and different reagents shows that the interaction occurs on equimolar basis via the formation of a charge transfer complex (1:1).

A more detailed examination was made for PX or TX complexes with the studied acceptors. The absorbance of the complex was used to calculate the association constant using the Benesi-Hildebrand equation[29].

$$[A_o]/A_{AD} = [1/\varepsilon_{AD}]-[(1/Kc_{AD}\,\varepsilon_{AD})\,(1/[D_o])]$$

Where $[A_o]$ and $[D_o]$ are the total concentration of the interacting species, A_{AD} and ε_{AD} are the absorbance and molar absorptivity of the complexes at their λ_{max}, and Kc_{AD} is the association constant of the complex. On plotting the value of $[A_o] / A_{AD}$ vs $1 / [D_o]$, a line was obtained with slope equals $(1/\varepsilon_{AD}. Kc_{AD})$ and intercept of this line with the ordinate is $(1/\varepsilon_{AD})$. The molar absorptivities are comparable with those obtained

from regression line equation of Beer's law.

Analytical data

A calibration graph was constructed usig a standard solution of PX or TX. Under the optimum experimental conditions, a linear relationship existed between the absorbance and concentration of the drug in the concentration ranges listed in TABLE 1. The correlation coefficients, intercepts and slopes of the calibration graphs are calculated using the least squares method. For more accurate analysis, Ringbom optimum concentration ranges are calculated and listed in TABLE 1.

The reproducibility and accuracy of the suggested methods were assessed[30] using different concentrations. The validity was checked occasionally during the work by running six replicate standard samples containing 15 μg ml^{-1}. At this concentration, the RSD% were = 1.8%.. The molar absorptivity and Sandell sensitivity are calculated and recorded in TABLE 1.

Comparison of recoveries obtained with the proposed methods (99.5 ± 1.1%) with the purity of PX and TX as determined according to the official methods[24].

(99.2±1.8 %) showed a high accuracy of the proposed methods. The proposed methods are simpler, less time consuming and more sensitive than the official

TABLE 2 : Determination of PX in pharmaceutical formulations applying the standard addition technique.

Sample	Content mg	Taken mg	Added μg ml^{-1}	Found* μg ml^{-1}			
				TCNQ	TCN	DDQ	Official PB[24]
Capsules Feldene	10	4.0	-	3.97	3.98	4.02	3.93
			4.0	8.05	7.90	7.95	7.80
			8.0	12.1	12.1	11.9	11.7
			12.0	16.15	15.9	16.1	15.6
t-value [b]				1.27	1.53	1.38	
F-test [b]				2.68	3.15	2.81	
Feldene	20	10	-	10.05	10.1	9.93	9.82
			3	12.95	13.05	12.9	12.75
			6	16.1	15.95	16.05	15.7
			9	19.15	18.9	18.9	18.6
t-value [b]				1.63	1.28	1.44	
F-test [b]				3.42	2.56	2.97	
Tablets Feldene	10	5.0	-	4.98	5.03	4.96	5.07
			5	10.05	9.95	10.1	9.9
			10	15.1	14.9	14.95	14.8
			15	19.9	20.1	20.15	19.7
t-value [b]				1.09	1.59	1.25	
F-test [b]				2.33	3.21	2.78	
Suppositories Feldene	20	8	-	7.95	8.04	7.94	7.88
			5.0	13.1	12.95	12.9	12.75
			10	18.15	18.1	18.15	17.6
			15	22.9	22.85	23.20	22.5
t-value [b]				1.53	1.19	1.36	
F-test [b]				3.17	2.65	2.88	

***Average of six determination; aTheoretical t and F-values for five degrees of freedom and at 95% confidence level are 2.57 and 5.05 respectively.**

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TABLE 3 : Determination of TX in pharmaceutical formulations applying the standard addition technique

Sample	Content mg	Taken mg	Added μg ml^{-1}	Found* μg ml^{-1}			
				TCNQ	TCN	DDQ	Official[24]
Epicotil	20	12	-	11.94	12.05	12.1	12.2
			2.5	14.55	14.45	14.47	14.75
			5.0	17.1	17.15	16.9	17.35
			7.5	19.4	19.6	19.35	19.9
t-value [b]				1.49	1.17	1.66	
F-test [b]				3.08	2.61	3.42	
Epicotil	20	6	-	6.05	5.96	6.04	6.1
			6	12.1	12.05	11.95	12.2
			12	17.9	17.95	18.1	18.25
			15	21.25	20.9	20.85	21.35
t-value [b]				1.4	1.11	1.68	
F-test [b]				3.05	2.48	3.43	

***Average of six determination; aTheoretical t and F-values for five degrees of freedom and at 95% confidence level are 2.57 and 5.05 respectively.**

BP methods[24]. Moreover, the proposed methods could be used for the routine determination of PX and TX in bulk form or in pharmaceutical preparations.

Interferences

Regarding the interference of the exicipients and additives usually presented in pharmaceutical formulations interference due to the degradation products of the PX and TX, the energy of the charge transfer (E_{CT}) depends on the ionization potential (Ip) of the donor and the electron affinity of the acceptor (E_A), hence the λ_{max} values of the other π-donors mostly differ from that of the investigated compounds if they are able to form CT complexes. Preliminary experiments showed that all additives, excipients and degradation products did not form CT complexes with the acceptors under consideration, indicating the high selectivity of the proposed methods and applicability to use for routine assay in pure and in pharmaceutical preparations.

Analytical Applications

The proposed methods were successfully applied to various pharmaceutical preparations viz tablets, capsules and suppositories. The results shown in TABLES 2 and 3 are statistically compared with the official BP methods[24]. For further confirmation, the standard addition method was applied to test the reliability and recovery of the proposed procedures, since the CT complexes are stable for 3 hrs. The recovery studies were carried out after adding known quantities of pure drug to the pre-analyzed formations. The percentage recoveries were found to be close to 100%, (TABLES 2 and 3), indicating no interference from all additives excepients that might be found in different formulations. Consequently, the methods are simple, rapid and stability indicating assay.

The results obtained were compared with those obtained using the official BP methods[24]. The accuracy via t-value and the assessment of precision via F-test for five degree of freedom and 95% confidence level were calculated and the results indicated that there is no significant difference between them, (TABLE 2).

CONCLUSION

The proposed methods are simpler, less time consuming and more sensitive compared to the official BP methods. The colour development at ambient temperature requires 60, 75 and 45 min. using TCNQ, TCNE and DDQ, respectively. This can be shortened to 10 min. on raising the temperature up to 70±1°C. The proposed method is suitable for the determination of PX or TX in pharmaceutical preparations without interference from additives and excipients such as starch and glucose or from common degradation products, sug-

gesting applications in bulk drug analysis.

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