

Use of 7,7,8,8-tetracyanoquinodimethane for spectrophotometric determination of certain local anaesthetics and procainamide hydrochloride

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Abstract: A simple and rapid spectrophotometric procedure has been developed for the determination of four local anaesthetics containing a free primary amine moiety and of procainamide hydrochloride as the drug substances and in dosage forms. The method is based on the reaction of the drug with 7,7,8,8-tetracyanoquinodimethane in alkaline solution to produce yellow products. The chromogen was measured at 473 nm. The effects of several variables on colour development were established. Job's plots of absorbance versus molar ratio of drug to reagent indicated a 1:1 ratio for all the drugs studied. Results of the analysis of drug substances and their dosage forms by the proposed method are in good agreement with those obtained by the USP XX method.

Keywords: *Spectrophotometry; local anaesthetics; anti-arrhythmic drugs; 7,7,8,8-tetracyanoquinodimethane; pharmaceuticals.*

Introduction

Benzocaine, procaine hydrochloride, butacaine sulphate and propoxycaine hydrochloride are widely used as local anaesthetics and are available as injections and ophthalmic and otic solutions. Procainamide hydrochloride is used to prevent atrial and ventricular arrhythmias; it is available as an injection and as tablets.

Several methods have been reported for the determination of the local anaesthetics studied and of procainamide hydrochloride. These methods include titrimetry [1-4], spectrophotometry [5-10], fluorimetry [12, 13] and chromatography [14-18]. Pharmacopoeial methods [1, 2] for these drugs include nitritometric and UV spectrophotometric procedures.

In this paper, a simple, rapid and accurate spectrophotometric method is reported for the determination of four local anaesthetics and procainamide hydrochloride as the drug substances and in some dosage forms. The proposed method involves the use of 7,7,8,8-tetracyanoquinodimethane (TCNQ) as the chromogenic reagent.

Experimental

Apparatus

A Zeiss spectrophotometer PM2DL (Zeiss, Oberkochen, FRG) and a Unicam SP 1750 UV-vis spectrophotometer (Pye-Unicam, Cambridge, UK) were used.

Materials

Pharmaceutically pure benzocaine, procaine hydrochloride, propoxycaine hydrochloride, butacaine sulphate and procainamide hydrochloride were donated by Merck (Darmstadt, FRG), Lederle (Wayne, NJ, USA), Specia (Paris, France) and Bailly and were used without further purification. Pharmaceutical preparations were obtained locally. All reagents and solvents were of analytical grade. Distilled water was used.

Standard solutions. Stock solutions of all drugs were prepared at a concentration of 1 mg ml⁻¹ in water except for benzocaine solution which was prepared in 0.1 M HCl. Further dilutions with water were made to give drug concentrations of 5-100 µg ml⁻¹.

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Procedure

To obtain the spectra and the relevant calibration curves, 1.00 ml of drug solution (5–100 $\mu\text{g ml}^{-1}$) was mixed with 1 ml of TCNQ solution (0.120% w/v TCNQ in acetonitrile) in a 10-ml volumetric flask; 1 ml of 0.15 M sodium carbonate was added and the solution was diluted to 10 ml with water. The absorbance was measured after 2 min at 473 nm against a reagent blank prepared similarly.

Analysis of pharmaceutical preparations.

Injections and solutions. A 1.00 ml volume of the mixed contents of 10 ampoules of procaine hydrochloride injection or 1 ml of butacaine sulphate solution was transferred by pipette into a 100-ml volumetric flask and diluted to volume with water; further dilutions with water were made to give drug concentrations of about 25 and 50 $\mu\text{g ml}^{-1}$ for procaine hydrochloride and butacaine sulphate, respectively. A 1.00 ml volume of this solution was used for the assay as described in the procedure.

Results and Discussion

Five primary aromatic amine compounds with different substituents, procaine hydrochloride, benzocaine, propoxycaine hydrochloride, butacaine sulphate and procainamide hydrochloride were found to react with TCNQ in an alkaline medium to produce intensely coloured products. The absorption spectra of the reaction products of all the drugs studied were more or less identical and exhibited maximum absorption (λ_{max}) at 473 nm, but with different absorptivities. Figure 1 shows the absorption spectra of procaine hydrochloride and benzocaine as examples.

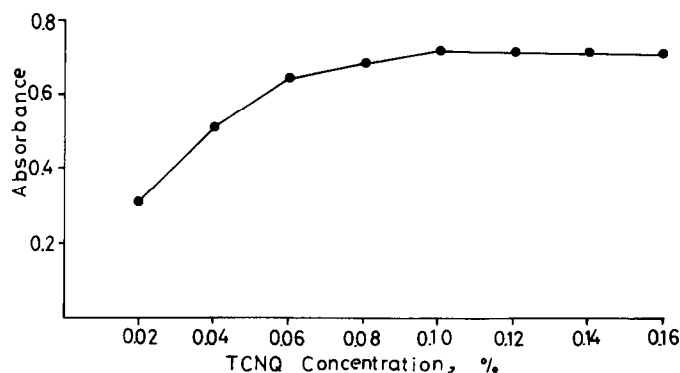


Figure 2 Effect of TCNQ concentration on the intensity of the coloured product with benzocaine, 2 $\mu\text{g ml}^{-1}$.

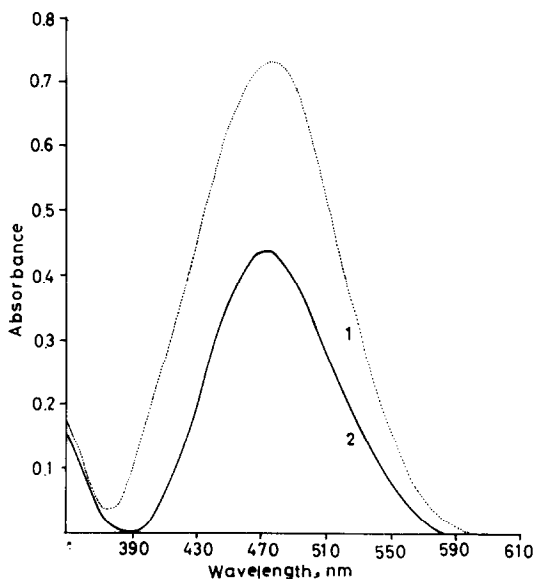


Figure 1 Absorption spectra of reaction products from TCNQ with (1) benzocaine, 2 $\mu\text{g ml}^{-1}$ and (2) procaine HCl, 2 $\mu\text{g ml}^{-1}$ in 0.15 M sodium carbonate.

Optimization of variables

Factors affecting the colour development, sensitivity and conformity with Beer's law were investigated with benzocaine as a model compound, since the other drugs behave similarly.

The effect of variation of the concentration of TCNQ was studied. The highest and most reproducible absorbance was obtained by using 1 ml of TCNQ solution (0.120% w/v in acetonitrile) (Fig. 2).

To investigate the effect of pH, the reaction was carried out in acid, alkali, and buffer solutions. No colour was produced upon the addition of acids. Maximum colour intensity was observed at pH >10 (Table 1). The

Table 1
Effect of pH on the formation and intensity of the coloured products of TCNQ with benzocaine ($2 \mu\text{g ml}^{-1}$) and procaine HCl ($2 \mu\text{g ml}^{-1}$)

Solution	pH	Absorbance at 473 nm	
		Benzocaine	Procaine HCl
0.1 M HCl	≈ 1.1	—	—
0.1 M Acetic acid	≈ 2.9	—	—
Buffer 1*	7	—	—
0.1 M NaHCO_3	8.3	0.156	0.133
0.1 M NaOAC	≈ 9.3	0.438	0.301
Buffer 1*	11	0.643	0.382
Buffer 2†	11	0.639	0.368
0.1 M Na_2CO_3	11.5	0.691	0.410
0.1 M NaOH	≈ 12.9	0.650	0.372

*Theorell and Stenhagen buffer, pH 2.0–12.0 [22].

†Kolthoff and Vleeschhouwer buffer, pH 9.2–11.0 [22].

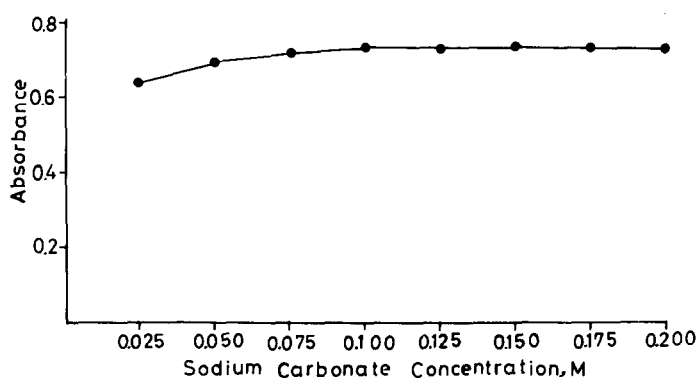


Figure 3

Effect of sodium carbonate concentration on the intensity of the coloured product of TCNQ with benzocaine, $2 \mu\text{g ml}^{-1}$.

absorbance, however, depended on the nature of the buffer system. The most stable and reproducible colours were obtained by using sodium carbonate. Figure 3 shows that the absorbance was maximal and constant when 0.1–0.2 M sodium carbonate was used. Use of 1 ml of 0.15 M sodium carbonate was recommended from the results of this study.

The colour was produced at room temperature and consequently there was no need to heat the reaction mixture. Maximum absorption was obtained within 2 min and remained stable for about 1 h.

The solvents studied were water, methanol, ethanol, isopropanol, acetone, dioxane, dimethyl sulphoxide, dimethylformamide and acetonitrile. The use of water as the dilution solvent afforded maximum stability and colour intensity (Table 2). In addition, water has a fairly good solvating power for the reaction mixtures.

Under these optimum conditions, a linear correlation was found between the absorbance

Table 2

Effect of dilution with different solvents on the colour intensity of the developed reaction products of TCNQ with benzocaine ($2 \mu\text{g ml}^{-1}$) and procaine HCl ($2 \mu\text{g ml}^{-1}$)

Solvent	Absorbance (λ_{max} , nm)	
	Benzocaine	Procaine HCl
Water	0.700 (473)	0.413 (473)
Methanol	0.509 (480)	0.293 (480)
Ethanol*	0.570 (483)	0.332 (483)
Isopropanol*	0.566 (485)	0.330 (485)
Acetone*	0.580 (510)	0.349 (510)
Dioxane†	0.050 (490)	0.052 (490)
Dimethyl formamide*	0.600 (508)	0.354 (508)
Dimethyl sulphoxide*	0.520 (508)	0.324 (508)
Acetonitrile	0.541 (500)	0.320 (500)

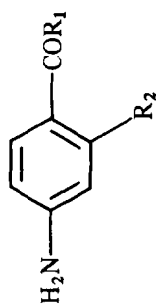
* A precipitate was formed and the colour was measured after filtration or centrifugation.

† Unstable colour.

for each drug and concentration in the range given in Table 3. Separate determinations at different concentrations of each drug gave relative standard deviations not exceeding 2%.

Table 4 shows the results obtained from the determination of procaine hydrochloride and

Table 3
Statistical data and absorption characteristics of the studied drugs



Drug	R ₁	R ₂	$\epsilon_{\max} \times 10^{-4}$ at 473 nm	Linear calibration range ($\mu\text{g ml}^{-1}$)	Intercept	Slope	Correlation coefficient (r)	Purity (% \pm SD)
Benzocaine	-O.C ₂ H ₅	H	5.81	0.4-4.0	-0.0099	0.3526	0.9960	99.3 \pm 1.2*
Procaine HCl	-O.(CH ₂) ₂ N(C ₂ H ₅) ₂	H	5.59	0.5-5.0	0.0152	0.2003	0.9976	100.3 \pm 1.0*
Butacaine sulphate	-O.(CH ₂) ₅ N	(CH ₂) ₃ CH ₃	5.10	1.0-20.0	0.0298	0.0687	0.9982	99.10 \pm 0.9†
Propoxycaine HCl	-O.(CH ₂) ₂ N(C ₂ H ₅) ₂	(CH ₂) ₃ CH ₃	2.72	1.0-15.0	0.0097	0.0813	0.9978	99.6 \pm 1.3*
Procainamide HCl	-NH(CH ₂) ₂ N(C ₂ H ₅) ₂	H	1.72	1.0-20.0	0.0081	0.0641	0.9992	98.80 \pm 0.7†

* According to USP 1985.

† According to USP 1980.

Table 4
Assay of dosage forms using the proposed method and the USP XX method

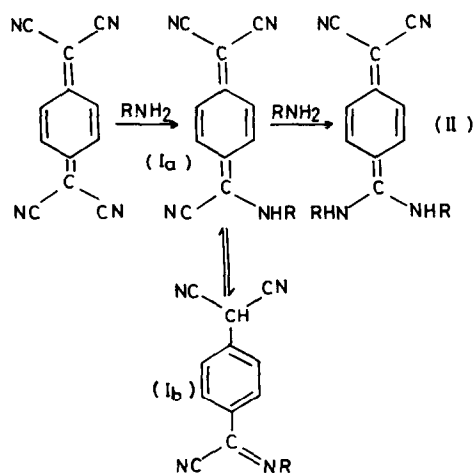
Formulation	Composition	Claimed	% found \pm SD ($n = 5$)	
			Proposed method	USP method
Procaine-adrenaline injection	Procaine HCl	2%	99.85 \pm 0.63	100.95 \pm 0.89
	Adrenaline	0.002%	$t = 2.256$; $F = 0.5012$	
Vera-Nova injection	Procaine HCl	40.6 mg/2 ml	96.54 \pm 1.85	97.31 \pm 1.11
	Caffeine	27.8 mg/2 ml	$t = 0.7981$; $F = 2.7780$	
Butacaine sulphate solution	Butacaine sulphate	100 mg/ml	96.56 \pm 1.20 $t = 0.4704$; $F = 0.7900$	96.95 \pm 1.35

butacaine sulphate in some of their dosage forms by the proposed method. The results were compared statistically with those obtained by applying the USP XX method and were found not to differ significantly.

The reaction of TCNQ with amine functions mostly proceeds via an addition-elimination mechanism [19], where the amine moieties displace one or two cyano groups from TCNQ to form the monoamine(I) or diamine(II), according to Scheme 1. The monoamine product may exist in two tautomeric forms (Ia and Ib).

The results obtained by studying the continuous molar variations of the studied drugs with TCNQ in the presence of 0.15 M Na_2CO_3 suggest the formation of monoamine reaction products. Investigations showed that the studied drugs interact with TCNQ in the ratio of 1:1 (Figure 4).

For all the studied drugs except procainamide HCl in USP 1985 [20], pharmacopoeial methods [1, 2, 20, 21] describe titrimetric



Scheme 1

procedures using sodium nitrite as a titrant; and end-point is detected externally by spotting on a starch-iodide paper or internally either by using ferrocypen as an indicator or amperometric by detection. For dosage forms,

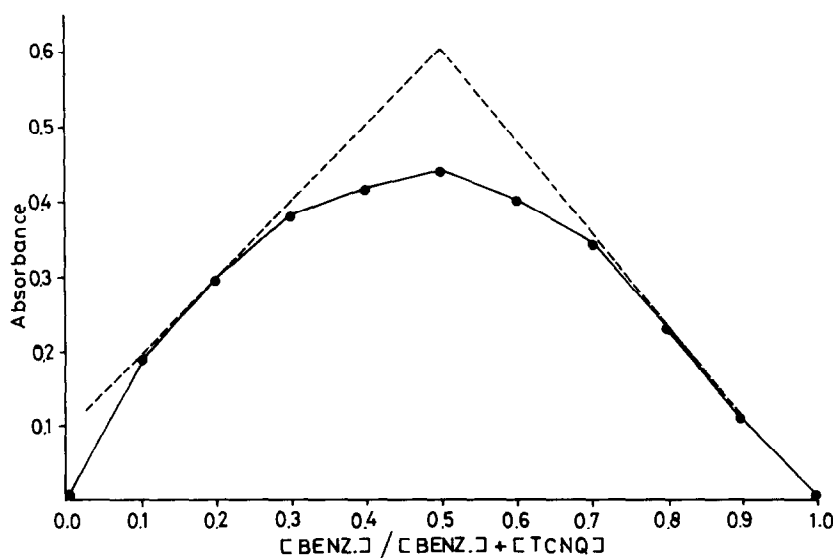


Figure 4
Continuous variation plot obtained from a solution of benzocaine (BENZ.) 1×10^{-3} M with TCNQ (1×10^{-3} M).

official methods mostly use either titrimetric procedures or direct UV measurements after many steps of extraction with chloroform.

Thus in comparison to the existing pharmacopoeial methods, the proposed method is simpler, more rapid, less laborious, cheaper and much more sensitive and accurate.

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