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Spectrophotometric determination of procainamide by the method of charge-transfer complexation with chloranilic acid

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Abstract

Spectrophotometric method of analysis of procainamide in its pharmaceutical dosage form by charge-transfer complexation using chloranilic acid was investigated. This investigation was designed to show that charge-transfer complexation can be a sensitive and accurate technique of assaying procainamide when compared to the official nitrite titration method. Procainamide in the presence of chloranilic acid spontaneously formed a purple complex with λ_{max} at 520 nm. The precision of the technique at various concentrations gave standard deviation range of between 0.70 and 0.85. The limit of detection and quantitation were 1.0×10^{-3} mg/ml and 3.0×10^{-3} mg/ml respectively. The complexation method obeys Beer-Lambert Law over a range of 1.0 mg/ml and 10 mg/ml concentrations and gave recoveries of 98.0 ± RSD 0.76 % as compared with 95.0 ± RSD 0.62 % by the Official sodium nitrite titration method. Statistical comparison of the two methods did not show any significant difference ($p>0.05$) in their levels of accuracy. The charge-transfer complexation technique is simple, sensitivity and accurate, therefore it can be used alongside the Official method of analysis of procainamide.

Keywords: Procainamide assay; Spectrophotometric analysis; Charge-transfer complexation

Introduction

Procainamide, [N-2-diethyl (amino ethyl)-p-aminobenzamide] is an effective and indeed the drug of choice in the treatment of cardiac arrhythmia. The presence of hydrolabile amino linkage in the molecule confers instability on this drug (Das Gupta, 1982).

The commonest dosage form is the tablet, in which stability or shelf life is retained for a considerable length of time if stored in airtight containers. Procainamide is hygroscopic and sensitive to light hence the need to assay it on a regular basis to ensure it quality assurance.

Amines and other functional groups with excess electrons engage in charge-transfer complexation (CTC) reaction with electron acceptors such as chloranil (Ayad *et al.*, 1984; Muralickrishna *et al*, 1984), tetracyanoethylene (Ibrahim *et al*, 1986) and chloranilic acid (Onunkwo and Adikwu, 1995; Adikwu and Echegi, 1997). Electron acceptors may be classified into π- and δ-acceptors. Quantitative analysis of many pharmaceutical and other nitrogenous

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compounds, using CTC has been reported extensively in the literature (Rizk *et al* 1981; Agrawal and El Sayed, 1981; Belal *et al.*, 1986; Sale and Askal, 1991; Abdel-Gawad, 1997; Ibrahim, 1997; Adikwu *et al.*, 1998).

These interactions have also been applied to the quantitative determination of carbonyl compounds after derivatization (Ayad *et al.*, 1985). Comparison of quantitative recoveries of a limited number of drugs by Pharmacopoeia methods and charge-transfer technique reported in the literature did not significantly differ from each other (Ibrahim *et al*, 1986; Belal *et al.* 1986; Sale and Askal, 1991; Ibrahim, 1997). Similarly, CTC has been applied to chromatographic (HPLC) separation of chiral molecules and in the separation/ detection of drugs on thin-layer chromatographic plates (Adikwu and Echegi, 1997; Onah, 1999) with a high degree of sensitivity.

Although charge-transfer interactions have proved very useful in the analysis of drugs with electron-donor groups, it is not without some limitations. For example, the possible formation of termolecular and isomeric complexes, specific solvation of the donor and acceptor molecules and the empirical determination of the optical absorption due to the complex may be affected by absorption of the reactants themselves (Foster, 1969). This situation is most common with weak complexes involving π-donors and π-acceptors, where only a small fraction of either component may be complexed. The principal disadvantages of CTC as a technique in quantitative analysis of basic drugs are the lack of direct evidence for the existence of the complex other than the colour formation. Secondly, the assumption inherent in the Benesi-Hildebrand model equation (Foster, 1969) when applied to CTC affects the reliability of data. In spite of these disadvantages, CTC is of scientific value (Ibrahim *et al*, 1986; Belal *et al.* 1986; Sale and Askal, 1991; Ibrahim, 1997) as an analytical tool.

Preliminary investigation in our laboratory has shown that procainamide and chloranilic acid form stable complexes in inert solvents whose uv/vis absorption obey the Beer-Lambert law. The stability of CTC underlies the suitability of the technique for analytical purposes, so the objective of this study is to evaluate quantitatively procainamide in its pharmaceutical dosage form. The suitability of this method with regard to quality assurance and stability-indicating assay will be discussed.

Experimental

Materials. Procainamide hydrochloride was purchased from Aldrich Chemical Company, (Missouri, USA) and was used without further purification. Chloranilic acid (Riedel de Haen, Germany) was recrystallized from acetone (M & B, UK). Chloroform (M & B, UK) was re-distilled and dried with anhydrous sodium sulphate (BDH, UK) to provide anhydrous chloroform. 1,4-Dioxan (BDH, UK) was also dried with anhydrous sodium sulphate and used without further re-distillation. All other reagents used were of analytical grade and were prepared fresh each time.

Determination of absorption maximum for chloranilic acid and its complex. Chloranilic acid solution (5.0×10^{-3} M l^{-1}) was prepared in 1,4-dioxan and its absorption maximum established using Spectronic 20 (Eagle Scientific, UK). Similarly, 5.0 ml of chloranilic acid and 5.0 ml of procainamide (5.03×10^{-2} M l^{-1}) dissolved in chloroform was mixed and the wavelength of the maximum absorption of the complex was determined. Equal volumes of chloroform and 1,4- dioxan served as reference solution.

Determination of extinction coefficient. Procainamide solution equivalent to 5.0×10^{-2} M was prepared in chloroform. Serial dilutions (1.0, 1.5, 2.0 and 2.5 ml) were transferred to 10 ml volumetric flasks and 1.0 ml of chloranilic acid added (5.0×10^{-3} M) added to each flask. The solutions were made up to volume with 1,4-dioxan. Four replicate preparations were made for each serial volume of procainamide measured. The contents were thoroughly mixed and their absorbances measured after 15 minutes. The absorbances were further monitored at intervals of 1 h for 6 h. The means of the absorbances and standard deviations were calculated and plotted against the concentrations of procainamide. The curve was regressed by the method of least squares (Bauer, 1971). The slopes gave the measure of the molar extinction coefficient.

Optimization of analytical technique. Different concentrations of procainamide were prepared and absorbances analyzed for precision, accuracy, selectivity, specificity and linearity. By reducing the concentration of the complex until minimum deflection was recorded, the limit of detection of the spectrophotometer was determined. Similarly, the limit of quantitation was established by determining the minimum concentration of the complex that gave a reproducible absorbance. Linearity of the relationship between concentration and absorbance was also established with different concentrations of the complex. The optimum concentration of chloranilic acid and procainamide were determined by keeping the concentration of one constant while varying the other and vice-versa, and the concentrations were further varied simultaneously. From these observations, the concentrations of procainamide and chloranilic acid that gave the best reproducibility were selected for the analysis.

Construction of calibration curve and its validation. Pure procainamide drug (5.03×10^{-2} M) was prepared in chloroform and choranilic acid (5.0×10^{-3} M) in 1,4-dioxan both in calibration flasks. Serial dilutions of the procainamide solution, were quantitatively transferred to 10-ml volumetric flasks and 1.0 ml of chloranilic acid added. Quintuplet preparations were made for each volume measured. The contents were thoroughly mixed and their absorbances read after standing for 15 minutes. Equal volumes of chloroform and 1,4-dioxan were contained in the reference cell. The solutions were kept in the dark and their absorbances further monitored at regular intervals of 24 h for 72 h. The means and standard deviations of the absorbances were calculated and plotted against the concentrations. The curve was regressed by the method of least squares (Bauer, 1971).

The calibration curve was validated by employing the procedure described above, except that different concentrations of procainamide were used. The mean absorbance for the repeatedly spiked concentrations of procainamide was measured and then converted to the corresponding concentrations using the calibrated curve. The percentage recovery and the errors with regard to the calibration curve were determined by comparing the concentration obtained from the calibration curve with actual concentration of the procainamide that was spiked. The relative standard deviation expressed in percentage for each concentration was calculated.

Assay of procainamide in tablet formulation. Procainamide tablets (purchased from the Jos University Teaching Hospital) equivalent to 0.5 g was powdered, dissolved in water and extracted with chloroform (25 ml x 3) and the volume was made up to 100 ml with chloroform. Serial dilutions were transferred in triplicates to 10-ml volumetric flasks and 1.0 ml of chloranilic acid (5.0×10^{-3} M) added to each flask. The contents were mixed and their absorbances determined at the absorption maximum. With the aid of standard curve, the percent recovery from the commercial tablets was calculated. Sodium nitrite titration of the procainamide was similarly carried out by the method described in the British Pharmacopoeia (BP 1993).

Results and Discussion

The absorption profile of chloranilic acid and its complex with procainamide is shown in Fig.1. The result obtained by the technique of donor-acceptor complexation and the official sodium nitrite method is similarly shown in Table 1. It can be observed that the proposed method gave more accurate result (mean recovery of 97.99 ± SD 0.738 %) than the nitrite titration method (mean recovery of 94.88 0.640 %).

The solution of chloranilic acid in 1,4-dioxan absorbed maximally at 420 nm (Fig.1), while that of the complex absorbed maximally at 520 nm (Fig.1). The solution turned purple instantaneously in the presence of procainamide, indicating that procainamide-chloranilic acid complex (λ_{max} = 520 nm) was formed. The formation of a purple colour is sufficient evidence that CTC was formed since neither procainamide nor chloranilic acid singly showed this appearance originally in solution. The purple colour was stable over a period of 72 h, therefore, suggesting that the complex was also stable during the 45 minutes of analysis. The choice of chloroform formed part of the optimization studies since chloroform does not form a complex with chloranilic acid. The purple colour has an extinction coefficient of 5797 l mol^{-1} cm^{-1}

The precision of this analytical procedure, tested at different concentrations, gave a range of standard deviation between 0.70 and 0.85 (Table 1) for the complexation method. Recovery experiments on the pure procainamide gave 100 %, while the pharmaceutical formulation gave values between 95 % and 98 % and with relative standard deviations of between 0.6 % and 0.8 % respectively (Table 1). These values indicate high degree of accuracy for the technique.

The limit of detection (LOD) of procainamide in the complex was 1.0 x 10^{-3} mg / ml, while the limit of quantitation (LOQ) was 3.0 x 10^{-3} mg / ml with a relative standard deviation of 1.102 %. Linearity tests at different concentrations of the analyte were similarly investigated. The following equation generally satisfied the mathematical model

$$A_{520} = 0.193x + 0.00952$$

where A_{520} is absorbance, x the concentration of procainamide in the final assay medium in moles per litre. Linear regression analysis by the method of least squares (Bauer, 1971) gave a coefficient of 0.97.

Recovery experiment of commercial procainamide tablets was carried out following the validation of the calibration curve. It was observed that the results were reproducible with low standard deviations (mean relative standard deviation = 0.74%) (Table1). Comparison of the charge-transfer technique with the official method was assessed through the calculation of the student's t-test. Considering the test at 95 % confidence limit, the calculated t-value was not significantly different from the theoretical value (Ayad *et al.* 1984; Rizk *et al.* 1981). It is reasonable, therefore, to conclude that there was no difference between the charge-transfer technique and the official method.

The principal limitation of the technique as applied in this investigation was our inability to establish quantitatively the identity of the assumed CTC. We observed that the complex exists only in solution (transient form) as infra-red spectrophotometry of the complex, when compared with that of procainamide and chloranilic acid, respectively, failed to exhibit any IR absorption shifts. This confirms the general observation that weak charge-transfer forces don't involve covalent bond types (Foster, 1969). The theoretical framework for these types of absorption has been elaborated (Foster, 1969).

Other limitations of analytical use of charge-transfer interactions include effect of solvent on the reactants; the intra-molecular charge-transfers between donors, termolecular and isomeric formation of products. Chloroform and ethers have been reported to form hydrogen bonding to aromatic π-donors in inert solvents (Foster, 1969). The optimization, in which various concentrations of procainamide were investigated without significant variation in standard deviation, suggests that the solvent effect, intramolecular, termolecular and isomeric forms were presumed absent or undetectable. Low concentrations of donors and acceptors, as employed in this investigation, have generally been reported to minimize these interactions (Foster, 1969). The nitrite titration of procainamide has serious limitations because the reaction must be carried out at sub-zero temperatures and the end-point detection can be difficult. Sodium nitrite titration method is certainly less sensitive than the spectrophotometric technique

This investigation has demonstrated that charge-transfer interactions can be used to quantitatively determine the actual concentration of procainamide in its pharmaceutical formulation. This technique offers the advantage of high sensitivity, uses less amount of material and is very simple.

Table 1: Recovery experiments by donor-acceptor complexation and sodium nitrite titration.

Procainamide tablets (250 mg)*	% Recovery: Complexation method (n=5)	% RSD	% Recovery: Nitrite method (n=5)	% RSD
1	97.81	0.814	94.29	0.581
2	99.50	0.655	94.55	0.701
3	97.50	0.691	94.90	0.686
4	98.29	0.789	95.04	0.590
5	97.99	0.849	95.10	0.727
6	96.98	0.634	95.45	0.557
Mean	97.99	0.738	94.88	0.640

*Label claim

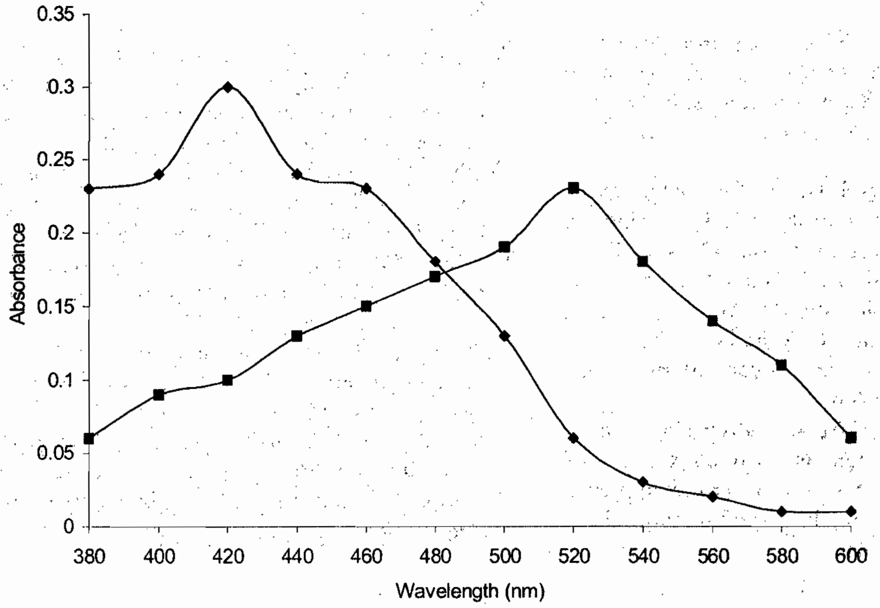

Fig. 1: Absorption profile for chloranilic acid (◆) and procainamide / chloranilic acid complex (■)

Conclusion

This study reveals that chloranilic acid-procainamide charge-transfer complex with its electronic transition in the visible region is more accurate, sensitive and simple method than the official technique in the analysis of procainamide and possibly other basic drugs. Molecular complexes that absorb in the visible region usually absorb very strongly and as a result, their absorbance can readily be determined. This technique requires that low concentrations of reactants be employed; the choice of solvents could reduce inter- and intra-molecular interactions and solvation of reactants and the analyte to the barest minimum.

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