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Use of π-Acceptors in Charge-Transfer Complexation of Some Cephalosporins: Chloranilic Acid as a Spectrophotometric Titrant in Non-Aqueous Media

AMIRAH SALEH AL-ATTAS
Department of Chemistry, College of Education Girls, P.O. Box 16531
Jeddah-21474, Kingdom of Saudi Arabia
E-mial: amirh-alattas@hotmail.com

A spectrophotometric titration method is described for the determination of some cephalosporins and their dosage forms using 0.005 M chloranilic acid solution in 1,4-dioxane as the titrant. The end-points is determined by measuring the change in absorbance of the sample at 488-528 nm. Quantitative recoveries with good reproducibility are reported for cephalexin, cefadroxile, cefaclor, cefuroxime, cefoxitin, ceftazidine and ceftriaxone eight dosage forms. The least-squares method for the end point location in the spectrophotometric titration is also proposed.

Key Words: Spectrophotometric titration, Cephalosporins determination, Chloranilic acid, Charge-transfer complexation.

INTRODUCTION

The existence of chloranilic acid (CA) in three different forms depending on the pH[1,2] of the solution, led to it's importance in the pharmacological analytical uses. Silifkin *et al.*[3] studied the nature of chloranilic acid complexes with amino acids and showed that 1:1 complexes are formed in solution and 1:2 complexes in the solid state. Habeeb *et al.*[4,5] studied the hydrogen bonded and proton transfer complexes of chloranilic acid with a series of nitrogen and oxygen bases in the solid state and in different solvents where 1:1 complexes produced. The utility of chloranilic acid as π-acceptor for the spectrophotometric determination of many drugs have been reported. El-sayed and Agrawal[6] introduced a spectrophptometric method to estimate some alkaloid through charge transfer complex formation with chloranilic acid. The determination of norfloxacin through charge transfer (CT) complex formation with chloranilic acid has been reported by Issa *et al.*[7]. Charan *et al.*[8] described a spectrophotometric method for estimating astemizole and laratadine through CT complex formation with chloranilic acid. Quina-pyramine dimethylsulphate which is a trypanocidal

drug was determined spectrophotometrically using chloranilic acid[9]. Some catecolamine drugs as pure and in various dosage forms were also determined spectrophotometrically with chloranilic acid[10]. Another applications of chloranilic acid for the determination of medical compounds containing basic nitrogen atoms have been described[11-14]. The molecular interaction between chloranilic acid (π-electron acceptor) and various electron donors are generally associated with the formation of intensely coloured chloranilic acid complexes which absorb radiation in the visible region. The photometric method based on these interaction are usually simple, rapid and convenient. All the above aspects encouraged us to make further studies utilizing chloranilic acid as π-acceptor complexing agent[15]. Hence, the aim of the current work is to spectrophotometric determination of some cephalosporins drugs through their charge transfer complex formation with chloranilic acid. These drugs are semisynthetic β-lactam antibiotics widely used in clinical chemotherapy in the pure and dosage forms[16].

EXPERIMENTAL

(UV/Vis) Spectrophotometer UV-1601 with personal spectroscopy software version 3.7-Shimadzu (Tokyo-Japan) was used. All chemicals and solvents used were of analytical reagent grade suppliers were as follows: cephalexin sodium and cefadroxile sodium (first generation), cefaclor sodium, cefuroxime sodium and cefoxitin sodium (second generation), cefotaxime sodium, ceftriaxone sodium and ceftazidine sodium (third generation) were obtained from Tabuk, Saudi Arabia Manufacturing Co).

For preparation of chloranilic acid solution (0.005 M), dissolve 1.045 g of *p*-chlorainlic acid in 1,4-dioxane and make up to 1 L. The solution, when stored in an amber glass bottle, was found to be stable for at least 6 weeks.

Pharmaceutical formulations: The following commercial dosage forms were subjected to the analytical procedure. Cephalex capsules (Tabuk, Saudi Arabia Manfacturing Co.) labeled to contain 250 mg cephalexin anhydrous per capsule. Droxil capsules (The united pharmaceutical Mfg. Co. Ltd.) labelled to contain 500 mg cefadroxil anhydrous per capsule and tabiclor capsules (Tabuk, Saudi Arabia Manfactaring Co.) labelled to contain 250 mg cefaclor anhydrous, Foxitin injection (Tabuk, Saudi Arabia Manfact uring Co.) labelled to contain 1 g cefoxitin sodium per injection, zinacef injection (Glaxo Saudi Arabia Ltd) lablled to contain 750 mg cerfuroxime, foxime injection labeled to contain 1 g cefotaxime sodium and triaxone injection lebelled to contain 1 g ceftriaxone sodium from (Tabuk, Saudi Arabia Manfacturing Co.).

Standard solution: Into a 20 mL calibrated flask, 5×10^{-3} M of each amino cephlosporins were prepared in methanol, then transferred to 100 mL calibrated flask in 1,4 dioxane to prepare 1×10^{-3} M from each drugs.

Working solutions: Capsules and injections: 5×10^{-3} M of each drugs from the contents of the injection or from a composite of the mixed contents of 20 capsules were prepared in methanol. Then follow the procedure as for the standard solutions.

General procedure: Transfer 5-25 mL of 1,4-dioxane solvent of each drugs into the titration flask. Deliver the titrant in 0.5 mL increments, stir and read the absorbance of the solution at 488-528 nm after each addition of titrant.

The end point is determined from a graph of absorbance *vs.* volume of titrant as being the intersection of the two straight line segments, or mathematically using the equation derived by the least-squares method[17].

RESULTS AND DISCUSSION

The amino cephalosporins cephalexin, cefadroxile, cefaclor, cefuroxime, cefoxition, cefoxitin, cefotaxime, ceftriaxone and ceftazidine have different structures and presented in Fig. 1. They exhibit different absorption maxima in the UV-Vis region[18]. However, with chloranilic acid in a non-aqueous medium these cephalosporins give apurple chromogen with almost similar maxima in the vicinity of 488-528 nm.

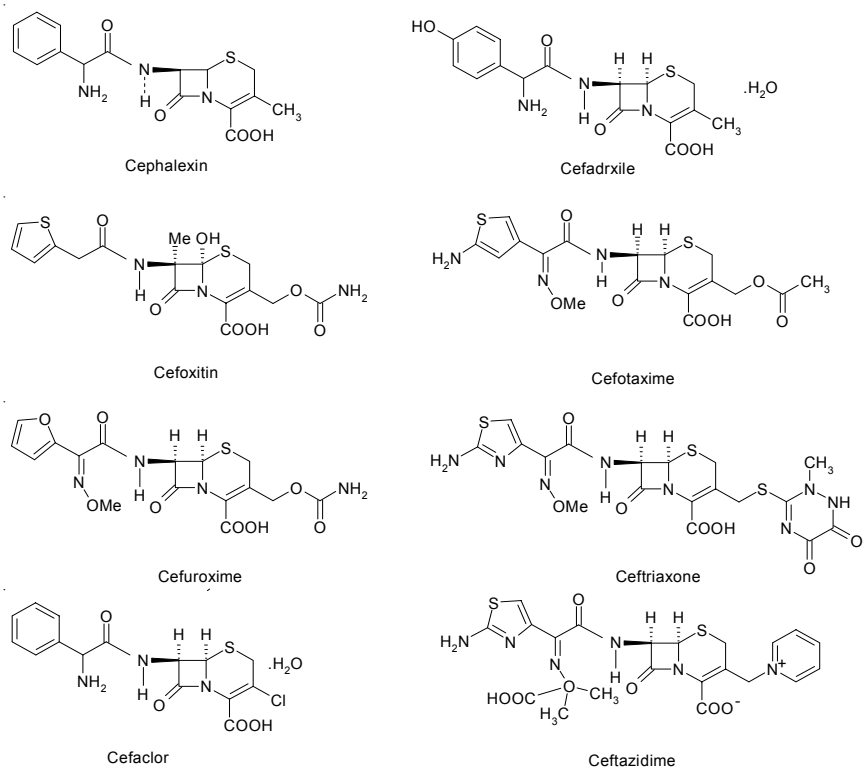
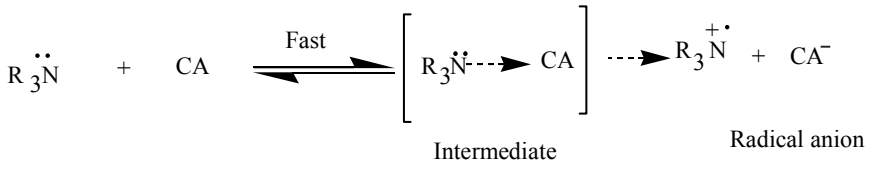


Fig. 1. Structures of cephalosporins

The identical nature of the absorption spectra, Figs. 2-4 are probably due to the common origin of the charge-tranfer complexation between the cephalosporins acting as π-donor and chloranilic acid acting a π-acceptor according to the following equation



1,4-Dioxane was used as the solvent owing to its low dielectric constant and it appears not to compete or shield the charge transfer process from donor to acceptor that is necessary for instant and stable formation at room temperature (about 25ºC). The electronic spectra were scanned against the same electron acceptor concentration to eliminate the possible overlap that may arise between CT complex band and that of the acceptor.

The operating wavelength of 488-528 nm was selected as it was the wavelength of maximum absorption for the complex and owing to the fact that there was no interference by other absorbing substances at this wavelength. The absorbance of the solution was corrected for dilution by multiplying the observed absorbance by the factor $(V_o + v)/V_o$ where V_o is the

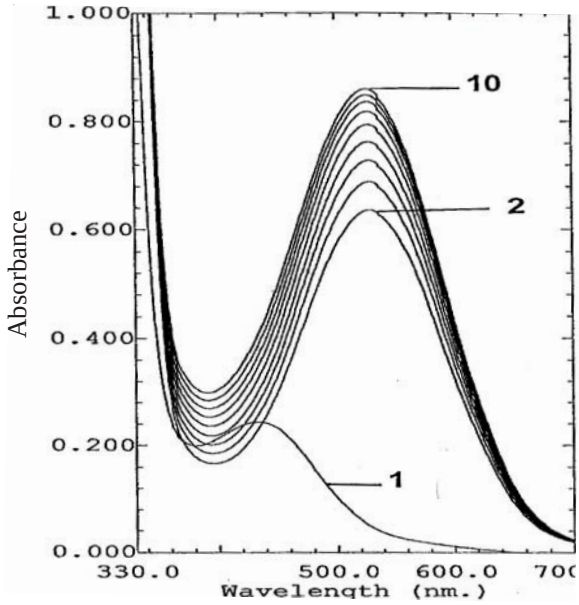


Fig. 2. Electronic absorption spectra of CT complex of ceftriaxone with CA in 1,4-dioxane, [CA] = 5×10^{-3} M, [ceftriaxone] = 0.0396, 0.403, 0.411, 0.417, 0.427, 0.435, 0.444, 0.453 and 0.462 mg/mL for curves from 2-10

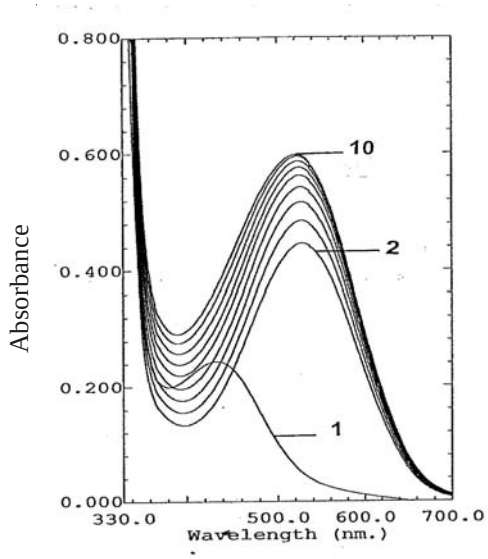


Fig. 3. Electronic absorption spectra of CT complex of cefoxitin with CA in 1, 4-dioxane [CA] = 5×10^{-3} M, [cefoxitin] = 0.305, 0.311, 0.317, 0.323, 0.329, 0.335, 0.342, 0.349, 0.356 and 0.364 mg/mL for curves from 2-10

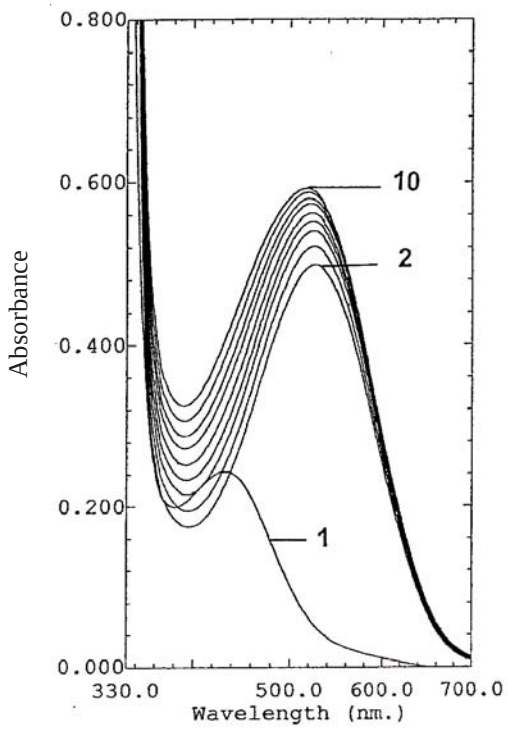


Fig. 4. Electronic absorption spectra of CT complex of cefroxime with CA in 1,4-dioxane, [CA] = 5×10^{-3} M, [cefroxime] = 0.303, 0.309, 0.314, 0.319, 0.326, 0.333, 0.339, 0.347 and 0.354 mg/mL for curves from 2-10

volume prior to the titrant addition and v is the volume of titrant added. Failure to make volume corrections may introduce unsuspected errors as extrapolation of a line incorrect slope will give an incorrect end-point.

Figs. 5-7 illustrate a typical titration graphs obtained with some cephalosporine drugs. Initially, there is an increase in absorbance owing to complex formation but after the equivalence point is reached there is very little further increase in absorption. An important conclusion from these figures is the formation of 1:1 molecular charge transfer complexes between the cephalosporins and chloranilic acid.

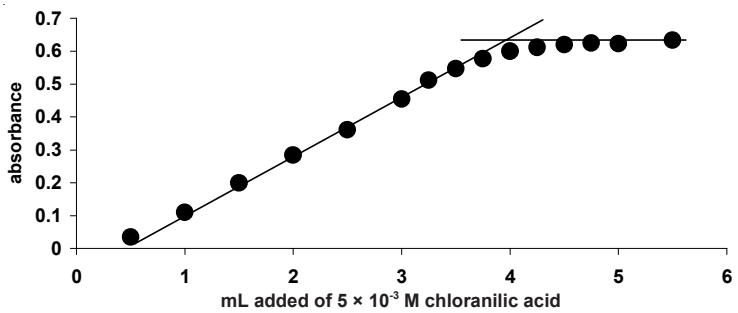


Fig. 5. Spectrophotometric titration curve of foxitin with 5×10^{-3} M CA at 527 nm

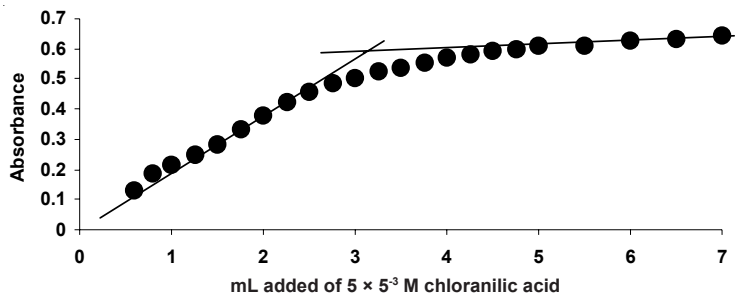


Fig. 6. Spectrophotometric titration curve of ceftazidine with 5×10^{-3} M CA at 524 nm

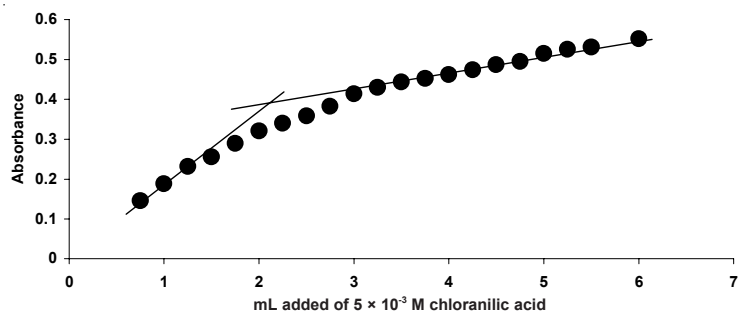


Fig. 7. Spectrophotometric titration curve of cephalexin with 5×10^{-3} M CA at 507.5 nm

Use of the least-squares method to determine the end point in spectorphotometric titration

In spectrophotometric titration graphs, where the graphical location of the end point may not be easy and subject to individual variation the following mathematical procedure based on the least squares method for location of the end-point was suggested[17].

The absorbance readings are measured not in the vicinity of the end-point but before and after the end-point and the absorbances should give a least-squares straight line. The regression line for the points located before the equivalence point can be described as

$$A_1 = a_1 + b_1 V_1 \quad (1)$$

and after the equivalence point, the regression equation for a given line is

$$A_2 = a_2 + b_2 V_2 \quad (2)$$

where A_1 and V_1 are the variable absorbances and volumes respectively, before the end-point and A_2 and V_2 the corresponding variables after the end point. The constants a_1 and b_1 are the intercept and the slope before the end-point, respectively and a_2 and b_2 the contstants after the end-point.

At the equivalence point these two lines intersect *i.e.*, $A_1 = A_2$ and $V_1 = V_2$. Therefore, equating the right hand side of eqns. 1 and 2:

$$a_1 + b_1 V = a_2 + b_2 V \quad (3)$$

Rearrangement gives

$$V = (a_1-a_2)/(b_2-b_1) \quad (4)$$

where V is the volume of titrant at the equivalence point

$$a_1 = \overline{A}_1 - b_1\overline{V}_1 ; \; a_2 = \overline{A}_2 - b_2\overline{V}_2$$

$$b_1 = n_1\Sigma V_1A_1 - \Sigma V\Sigma A_1/n_1\Sigma V_1^2 - (\Sigma V_1)^2$$

$$b_2 = n_2\Sigma V_2A_2 - \Sigma V_2\Sigma A_2/n\Sigma V_2^2 - (\Sigma V_2)^2$$

$\overline{A}_1, \overline{A}_2, \overline{V}_1$ and $\overline{V}_2$ are the corresponding mean values and n_1 and n_2 denote the number of absorbance measurements before and after the end-point.

Graphical and mathematical location of the end-point was applied to the data obtained in the spectrophotometric titration of each cephalosporins in Table-1.

The percentage recovery was calculated using the following equation:

$$\text{Recovery (\%)} = \frac{V \times \text{molarity of chloranilic acid} \times F}{\text{Mass of samples (g)}} \times 100$$

The factor F equals the relative molecular mass of cepholexin, cefadroxil, cefaclor, cefuroxime, cefoxitin, cefotaxime, ceftriaxone and ceftazidine, respectively.

TABLE-1
ASSAY RESULTS FOR SOME AMINO CEPHALOSPORINS USING GRAPHICAL AND LEAST SQUARES METHODS FOR THE LOCATION OF END-POINT. THE FIGURES IN PARENTHESES ARE THE MEAN PERCENTAGE RECOVERY ± STANDARD DEVIATION (SD)

Cephalosporins compounds	Amount taken (mg)	Volume (mL)	Graphical method recovery (%)	Volume (mL)	Least-squares method recovery (%)
Cephalexin	1.74	1.97	98.50	1.9850	99.25
	5.21	2.98	99.33	2.9990	99.97
X ± S.D.			(98.92 ± 0.59)		(99.61 ± 0.51)
			2.75†		1.08†
Cefadroxil	3.81	1.97	98.50	1.9934	99.67
	5.72	2.99	99.67	3.0100	100.33
X ± SD			(99.09 ± 0.83)		(100.00 ± 0.47)
			1.54†		0.00‡
Cefaclor	1.91	0.99	99.00	0.9995	99.95
	3.81	2.01	100.00	1.9990	99.95
	5.72	2.99	99.67	3.0100	100.33
	9.54	5.02	100.40	4.9900	99.80
X ± SD			(99.89 ± 0.70)		(100.01 ± 0.23)
			0.31‡		0.08‡
Cefuroxime	2.12	0.99	99.00	0.9995	99.95
	4.24	1.99	99.50	2.0100	100.50
	6.37	3.03	101.00	2.9995	99.98
	10.61	5.02	100.40	5.0086	100.17
X ± SD			(99.98 ± 0.89)		(100.15 ± 0.25)
			0.045‡		1.2‡
Cefoxitin	2.14	0.97	97.00	0.9850	98.50
	4.28	2.05	101.50	1.9958	99.93
	6.41	5.97	99.00	3.0000	100.00
	10.69	4.99	99.80	4.9995	99.99
X ± SD			(99.33 ± 1.87)		(99.61 ± 0.74)
			0.74‡		1.05‡
Cefotaxime	2.28	1.01	101.00	1.0000	100.00
	4.56	2.01	100.50	1.9995	99.98
	6.83	2.98	99.33	2.9999	99.99
	11.39	5.01	100.20	5.0500	101.00
X ± SD			(100.26 ± 0.70)		(100.24 ± 0.51)
			0.72‡		0.94‡
Cetriaxone	2.77	0.98	98.00	1.0092	100.92
	5.55	1.99	99.50	2.0000	100.00
	8.32	2.01	100.50	2.9925	99.75
	13.86	4.98	99.60	4.9995	99.99
X ± SD			(99.40 ± 1.04)		(100.22 ± 0.47)
			1.15‡		0.89‡
Ceftazidine	2.72	1.01	101.00	0.9850	98.50
	5.47	2.02	101.00	1.9950	99.98
	8.20	2.99	99.67	2.9950	99.83
	13.67	4.988	99.60	5.0100	100.20
X ± SD			100.32 ± 0.79		(99.63 ± 0.77)
			0.75‡		0.97‡

†Value for $t_{tcalculated}$ for which $t_{theoretical}$ = 3.30 for 2º of freedom at confidence interval = 95%.
‡Value for $t_{calculated}$ for which $t_{theoretical}$ = 2.78 for 4º of freedom at confedence interval = 95%.

Statistical comparison for graphical and mathematical location of the end-point for cephalosporins (Table-1) reveals that both methods are of equal accuracy, as none of the values for $t_{calcuated}$ exceed $t_{theoretical}$. The proposed method was applied for pharmaceutical preparations where both the calculated t and f values are not exceed the theoretical values as shown in Table-2.

TABLE-2
ASSAY RESULTS FOR SOME PHARMACEUTICAL PREPARTIONS

Preparations	Amount taken (mg)	Mean recovery* ± SD	Reported Method[19]
Cephalex (250 mg/capsule)	1.74-5.21	100 ± 0.67 t (0.9394), F (4.2359)	99.42 ± 1.38
Droxil (500 mg/capsule)	3.81-5.72	99.17 ± 1.27 t (0.2839), F (3.6484)	99.33 ± 0.67
Tabiclor (250 mg/capsule)	1.91-9.54	98.58 ± 0.82 t (2.1134), F (1.7559)	99.76 ± 1.09
Foxitin (1 g/injection)	2.14-10.69	99.44 ± 0.48 t (0.6879),F (10.0702)	98.99 ± 1.53
Zinacef (750/mg injection)	2.12-10.61	100.00 ± 0.99 t (2.2818), F (3.8048)	97.96 ± 1.95
Foxime (1 g/injection)	2.28-11.39	100.00 ± 0.50 t (2.2088), F (3.9683)	98.99 ± 1.00
Triaxone (1 g/injection)	2.77-13.86	100.47 ± 0.50 t (2.3097), F (5.9714)	98.44 ± 1.20

Number of determinations = 3.
*Percentage recovery in cephalosporins and percentage of lable claim in pharmaceutical preparation.

The use of the least-squares method in the location of the equivalence point is better than the graphical method as the certainty with which the end-point is determined is increased. As indicated above, the graphical method is subjective, as in some instance different lines can be drawn through the points on the graph giving different end-point, whereas only one end-point is obtained by the least squares method.

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