Spectroscopic Analytical Study for the Charge-Transfer Complexation of Certain Cephalosporins with Chloranilic Acid

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Studies were carried out to develop a simple, rapid and accurate spectrophotometric method for the analysis of fifteen cephalosporins. The method depends on the charge-transfer complexation reaction between any of these drugs as an n -electron donor and p -chloranilic acid (p -CA) as a π -acceptor to form a violet chromogen measured at 520 nm. Different variables affecting the reaction were studied and optimized. Under the optimum conditions, linear relationships with good correlation coefficients (0.9986 – 0.9996) were found between the absorbances and the concentrations of the studied drugs in the range of 4 – 1200 $\mu\text{g ml}^{-1}$. The limits of assay detection ranged from 2.54 to 42.83 $\mu\text{g ml}^{-1}$. The accuracy and precision of the method were satisfactory. The method was successfully applied to an analysis of the studied drugs in their pharmaceutical formulations; the recovery percentages ranged from $96.76 \pm 0.87\%$ to $100.50 \pm 1.30\%$. The interaction sites were confirmed by UV/VIS, IR, $^1\text{H-NMR}$ techniques. Molecular modeling for the interaction was used for deriving an equation for calculating the ϵ value for a particular drug. This equation gave a perfect prediction for the degree of interaction of the investigated drugs with the p -CA reagent.

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Introduction

By reviewing the available colorimetric procedures developed for the analysis of the first, second, and third-generation cephalosporins, namely cephradine, cephalexin, cefaclor, cefadroxil, cefprozil, cefixime, cephodoxime proxetil, ceftazidime pentahydrate, and the sodium salts of cephazolin, cephalothin, cephapirin, cephaloridine, cefotaxime, cefoperazone, and ceftriaxone, one can easily recognize that most of these methods involve the cleavage of the β -lactam moiety of the cephalosporins structure. These methods include spectrophotometric methods, such as a flow-injection analysis after heating with iron(III) and o -phenanthroline,¹ a flow injection analysis after reacting the liberated sulfide with N,N -diethyl- p -phenylenediamine and iron(III),² a reaction with potassium hexacyanoferrate,³ molybdophosphoric acid,⁴ chromotrope 2B and chromotrope 2R,⁵ glucitol and sodium hydroxide,⁶ NBS and NCS,⁷ copper(II) and vanadium,⁸ MBTH and 2,6-dichloroquinone-4-chlorimide,⁹ oxidation with either iron(III) or cerium(IV),¹⁰ mercurochrome,¹¹ ferric hydroxamate,¹² tetrachloroquinone,¹³ p -benzoquinone and tetrachlorobenzoquinone,¹⁴ ammonium molybdate,⁵ ammonium vanadate,¹⁶ sodium nitroprusside,¹⁷ NBD-Cl¹⁸ and tetrazolium salts.¹⁹ The pharmacopoeial methods for analyzing the investigated cephalosporins are either chromatographic methods,^{20,21} which are expensive, or titrimetric methods,²¹

which are less sensitive. Meanwhile, most of the other reported methods are lengthy and/or tedious.

A direct chemical analysis based on the reactivity of the intact molecule without its cleavage is not frequently encountered. The methods that are based on charge-transfer complexation are usually rapid and simple to perform. In this respect, we previously described the utility of charge-transfer complexation reactions for the analysis of penicillins^{22–24} as well as some cephalosporins²⁵ with other acceptors. The present work describes an improved direct simple analytical procedure that can be applied at quality-control laboratories for the analysis of cephalosporins. This analytical procedure was based on the reactivity of an intact molecule without cleavage. The chemical structures of the investigated cephalosporins are given in Table 1.

Experimental

Apparatus

Shimadzu UV 1601 (Tokyo, Japan) and Spectronic 21D (Tokyo, Japan), UV-visible spectrophotometers, both with 1-cm quartz cells, were used. An infrared spectrometer (IR-470, Shimadzu, Japan) and a $^1\text{H-NMR}$ spectrometer (EM-360, 60 MHz NMR spectrometer USA, Varian Instrument Division, Palo Alto, CA) were also used.

Materials, reagents and dosage forms

Cefazolin sodium, cefprozil anhydrous, and cephapirin sodium (Bristol Myers-Squibb Pharmaceutical Co., Cairo, Egypt), cefoperazone sodium (Pfizer Co., Egypt), cefaclor and

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Table 1 Chemical structures of the investigated cephalosporins

R ₁	R ₂	R ₃	R ₁	R ₂	R ₃
1 Ceftazidime pentahydrate		H	9 Cephalothin sodium		H
2 Cephalexin monohydrate		H	10 Cefixime		H
3 Cephodoxime proxetil			11 Cefprozil anhydrous		H
4 Cefotaxime sodium		Na	12 Cephaloridine sodium		Na
5 Ceftriaxone sodium		Na	13 Cefoperazone sodium		Na
6 Cefadroxil monohydrate		H	14 Cefazolin sodium		Na
7 Cefaclor		H	15 Cephapirin sodium		Na
8 Cephadrine		H			

cephadrine (Sigma Chemical Co., St. Louis, USA), ceftazidime pentahydrate and ceftriaxone sodium (T3A Pharma Group, Assiut, Egypt), cephalexin monohydrate (Arab Drug Co., Cairo, Egypt), cefotaxime sodium (CID, Cairo, Egypt), cefadroxil monohydrate (Amoun Pharmaceutical Industries Co., APIC, Cairo, Egypt), cephodoxime proxetil, and cephalothin sodium (Hoechst Marion Roussel, S. A. E., Cairo, Egypt), cephaloridine sodium (Eli Lilly & Co., Indianapolis, USA), and cefixime (El-Hekma Co., Cairo, Egypt) were obtained and used without purification. 2,5-Dichloro-3,6-dihydroxy-1,4-benzoquinone (*p*-chloranilic acid, *p*-CA, BDH Chemicals, Poole, UK) 5 mg ml⁻¹ in acetonitrile, was prepared fresh daily. All solvents used were of analytical-reagent grade.

Pharmaceutical formulations

The available commercial dosage forms subjected to the analysis by the proposed procedure are given in Table 2.

General analytical procedures

Aliquot volumes of standard stock solutions containing 0.04 – 12 mg drug were transferred into 10-ml calibrated flasks. One milliliter of the *p*-CA solution (4 mg ml⁻¹ in acetonitrile) was added, and the reactions were allowed to proceed for 5 min at room temperature (25 ± 5°C), and then diluted to volume with acetonitrile. The absorbances of the resulting solutions were measured at the wavelength of maximum absorption (520 nm) against reagent blanks treated similarly.

Table 2 Commercial cephalosporins-containing dosage forms

Product	Manufacturer	Active material	Content/mg
Fortum® vials	GlaxoWellcome, S.A.E., Cairo, Egypt	Ceftazidime·7H ₂ O	250
Cephalexin® capsules	Army Factory of Drugs, Cairo, Egypt	Cephalexin·H ₂ O	250
Ceporex® tablets	GlaxoWellcome, S.A.E., Cairo, Egypt	Cephalexin·H ₂ O	250
Clafuran® vials	Hoechst Orient, S.A.E., Cairo, Egypt	Cefotaxime Na	500
Cefotax® vials	T3A Pharma Group, Assiut, Egypt	Cefotaxime Na	1000
Velosef® capsules	Bristol Myers-Squibb Pharmaceutical	Cephadrine	250
Velosef® vials	Co., Cairo, Egypt (for capsules, vials, and suspension)		250
Velosef® suspension			125
Cefzil® tablets	Bristol Myers-Squibb Pharmaceutical Co., Cairo, Egypt	Cefprozil anhydrous	250
Cefobid® vials	Pfizer Egypt, S.A.E., Cairo, Egypt	Cefoperazone Na	500
Totacef® vials	Bristol Myers-Squibb Pharmaceutical Co., Cairo, Egypt	Cefazolin Na	500
Cefatrexyl® vials	Bristol Myers-Squibb Pharmaceutical Co., Cairo, Egypt	Cephapirin Na	500

Analysis of tablets and capsules

Twenty tablets or the contents of 20 capsules of each drug were weighed and powdered or evacuated, and a quantity of the powder equivalent to 100 mg was transferred into a 50-ml calibrated flask, dissolved in 2 ml of methanol, swirled and sonicated for 2 min. The solutions were mixed well by shaking for 15 min, and then completed to volume with acetonitrile. The solutions were filtered; the first portion of the filtrate was rejected. The filtrates were quantitatively diluted with methanol to yield concentrations in the linear range of the assay of each particular drug.

Analysis of vials and suspensions

A quantity of powder equivalent to 100 mg was transferred into a 50-ml calibrated flask, dissolved in 2 ml of methanol, and then manipulated as described under the analysis of tablets and capsules starting from "swirled and sonicated...".

Determination of molar ratio

The Job's method of continuous variation²⁶ was employed. Master equimolar solutions of drug and *p*-CA were prepared. The concentrations of these solutions were 4.71×10^{-3} M (for cefazolin sodium, cefoperazone sodium, ceftazidime pentahydrate, ceftriaxone sodium, cephalothin sodium, cephalixin sodium, cephradine, and cefotaxime sodium), and 4×10^{-2} M (for cefixime, cefprozil anhydrous, cefaclor, cephalexin, cefadroxil monohydrate, cephaloridine sodium, and cephradine proxetil). A series of 10-ml portions of the master solutions of drug with the *p*-CA reagent were made up comprising different complementary proportions (0:10, 1:9, ..., 9:1, 10:0) in 10-ml volumetric flasks. After the reactions were allowed to proceed for 5 min at room temperature ($25 \pm 5^\circ\text{C}$), the absorbances of the solutions were measured at 520 nm against reagent blanks.

Preparation of the complexes for infrared measurements

To 2 ml of 0.05 M methanolic drug solution, 2 ml of 0.05 M of CA in acetonitrile was added in a round-bottom flask containing ~30 ml of acetonitrile and stirred for 30 min. Acetonitrile was evaporated under reduced pressure, and the resulting residues were dried over calcium chloride.

Solutions for ¹H-NMR measurements

A 30 mg quantity of each drug was dissolved in 1 ml of DMSO-*d*₆, and 1 ml containing an equimolar amount of *p*-CA in the same solvent was added and used directly for ¹H-NMR measurements.

Results and Discussion

Absorption spectra

A chloranilic acid (*p*-CA) solution in acetonitrile displayed a maximum absorption peak (λ_{max}) at 435 nm, while the investigated cephalosporins showed negligible absorption in the 400 – 700 nm region. Mixing the solutions of any of the investigated drugs to a solution of chloranilic acid in acetonitrile resulted in a change of the yellowish-pink color of the *p*-CA to a purple color. As a consequence, the absorption band of *p*-CA showed a bathochromic shift, λ_{max} at 520 nm (Fig. 1, cephalixin was presented as a representative example). This was attributed to charge-transfer complex formation between the studied drugs and *p*-CA.

Reaction mechanism

The interaction of any of the investigated compounds with *p*-CA in polar solvents, such as acetonitrile or methanol, was a charge-transfer complexation reaction between the n-donors cephalosporins and the π -acceptor (*p*-CA), followed by the formation of a radical anion. Complete electron transfer from the donor to the acceptor moiety took place with the formation of intensely colored radical ions with high molar absorptivity values, according to the following scheme:

Chloranilic acid (*p*-CA) exists in three ionic forms, the neutral yellow-orange H₂A at very low pH, the dark purple HA[−] which is stable at pH = 3 and a pale violet A^{2−}, which is stable at high pH; these transformations are illustrated in the following scheme:

Since the reaction product in acetonitrile was purple, we might conclude that HA was the form of *p*-CA involved in the reaction described herein.

Optimization of reaction conditions

In order to select the most appropriate solvent for optimum reaction development and dilution, different solvents were tested. The results (Table 3) showed small shifts in the position

Fig. 1 Absorption spectra of cephalirin sodium, 75 $\mu\text{g ml}^{-1}$ (1), *p*-CA, 4 mg ml^{-1} (2), and their reaction product (3).

of the maximum absorption peak. The absorption intensities were also influenced. Acetonitrile was used throughout this work because it gave the highest absorbance readings. The results of the *p*-CA reagent concentration variation indicated that 1 ml of 4 mg ml^{-1} was optimum. The optimum reaction time was determined by following the color development at room temperature ($25 \pm 5^\circ\text{C}$). Complete color development was attained instantaneously with all the investigated compounds, and the color remained stable for at least a further 24 h. Although the charge-transfer complex was formed instantaneously, constant absorbance readings were obtained after not less than 5 min of standing at room temperature ($25 \pm 5^\circ\text{C}$).

Validation of assay procedures

Concentration ranges and linearity. At fixed optimum experimental conditions, the calibration curves for the investigated drugs with *p*-CA were constructed. The least-squares method was used to derive the regression equations for the proposed procedures (Table 4). Beer's plots ($n = 6$) at 520 nm revealed very small intercepts (0.0014 – 0.0626) and good linear relationships (correlation coefficients, $r = 0.9986 - 0.9996$) between the absorbance and the drugs concentrations over the range of 4 – 1200 $\mu\text{g ml}^{-1}$. The linearity of the assay was checked by a *t*-test for the intercepts and an analysis of the variance of the slopes.

Sensitivity and precision. The sensitivity of the proposed method was evaluated by determining the limits of detection (LOD), defined as 3SD of the intercept. The LOD values ranged from 2.54 to 42.83 $\mu\text{g ml}^{-1}$ in the case of cephalirin sodium and anhydrous cefprozil, respectively. In all of the drugs studied, the mean of six replicate analyses of solutions of the studied cephalosporins within the linear range listed in Table 4 gave coefficient of variations of up to 1.8%. This level of precision of the proposed method is adequate for a quality-control analysis of the studied drugs.

Specificity and interference. The proposed procedure was performed in the visible region away from the UV-absorption region of the investigated drugs (228 – 300 nm),²⁷ and the UV-absorbing interfering materials that might be coextracted from dosage forms. Potential interference by reducing sugars in the tablets, or powder for oral suspension was eliminated by extraction with methanol prior to analysis. However, interference was observed from L-arginine formulated with cephradine in Velosef vials. Therefore, the proposed procedure could not be used for the analysis of cephradine in the presence

Table 3 Effect of solvents on the position and intensity of absorption of the reaction of cephalirin sodium (45 $\mu\text{g ml}^{-1}$) with *p*-CA (4 mg ml^{-1})

Solvent	λ_{max}	Absorbance
Carbon tetrachloride	520	0.287
1,4-Dioxane	520	0.249
Benzene	533	0.310
Toluene	536	0.272
Xylene	520	0.249
Diethylether	530	0.283
Chloroform	520	0.374
Methylene chloride	530	0.400
1-Butanol	525	0.349
Propan-2-ol	530	0.358
Propan-1-ol	525	0.372
Acetone	523	0.456
Ethanol	525	0.373
Methanol	528	0.373
Dimethylformamide	524	0.249
Acetonitrile	520	0.525
Dimethylsulfoxide	532	0.131

of L-arginine, except after separation by a suitable separation technique.

Analysis of pharmaceutical formulations

The obtained high-intensity absorption bands and the very low reagent background made this procedure suitable for routine quality-control analyses of the investigated compounds with minimum interference. The proposed and official methods²⁰ were applied to the determination of the studied drugs in tablets, capsules, suspensions and vials containing different cephalosporins (Table 2). The obtained mean values ($\pm\text{SD}$) of the labeled amounts ranged from 96.76(± 0.87) – 100.50(± 1.30). In the *t*- and *F*-tests, no significant differences were found between the calculated and theoretical values at the 95% confidence limit of both the proposed and official methods (Table 5); this indicated similar precision and accuracy.

Stoichiometry of the reaction

Job's method of continuous variation²⁶ was used for determining the molar ratios of each of the investigated cephalosporins to the analytical reagent *p*-CA employed in the charge-transfer reaction. These ratios were 1:1 (in case of ceftazidime pentahydrate, cephalixin monohydrate, cefotaxime sodium, cephradine, cephaloridine sodium, and cefoperazone sodium), 1:2 (in case of cephradine proxetil, ceftriaxone sodium, cefadroxil monohydrate, cephalothin sodium, cefixime, cefprozil anhydrous, cefazolin sodium, and cephalirin sodium), and 1:4 (in case of cefaclor).

Infrared and ¹H-NMR studies

The formation of drug-*p*-CA charge-transfer complexes was confirmed by both IR and ¹H-NMR spectroscopy techniques. The majority of IR measurements on charge-transfer complexes have concerned shifts in the vibrational frequencies in a donor or acceptor (or both). Decreases in the vibration frequency of a particular band have been used as evidence for a particular site of a charge-transfer interaction.²⁸ The IR spectra of the complexes show some differences compared with the sum of the spectra of the two components. This was used to distinguish between weak charge-transfer complexes and the products of electron transfer or proton-transfer reactions.²⁸ The IR spectrum

Table 4 Quantitative parameters for the reaction of the studied cephalosporins with *p*-CA^a

Drug	Linear range/ μg ml ⁻¹	Intercept, <i>a</i> (t-calc.)	Slope, <i>b</i> (<i>s</i> ² <i>b</i>)	Corr. coeff. (<i>r</i>)	$\epsilon \times 10^3$ / dm ³ mol ⁻¹ cm ⁻¹	LOD/ μg ml ⁻¹	LOQ/ μg ml ⁻¹
1	20 – 200	0.0014(0.0036)	0.0037(0.0221)	0.9993	2.35	5.29	17.63
2	20 – 320	0.0070(0.0013)	0.0022(0.0429)	0.9996	0.75	5.37	17.90
3	80 – 1200	0.0626(0.0061)	0.0053(0.1570)	0.9991	0.33	31.27	104.22
4	40 – 400	0.0500(0.0080)	0.0018(0.0591)	0.9994	0.92	13.52	45.07
5	20 – 240	0.0130(0.0026)	0.0036(0.0386)	0.9990	2.12	13.94	46.47
6	20 – 320	0.0490(0.0089)	0.0027(0.0455)	0.9986	1.03	11.93	39.75
7	40 – 400	0.0600(0.0010)	0.0011(0.0519)	0.9994	0.84	21.60	72.01
8	12 – 144	0.0510(0.0002)	0.0068(0.0127)	0.9991	1.85	3.99	13.30
9	20 – 320	0.0290(0.0064)	0.0028(0.0337)	0.9995	1.11	6.16	20.53
10	100 – 1200	0.0110(0.0011)	0.0006(0.1524)	0.9986	0.28	34.37	114.57
11	40 – 480	0.0044(0.0707)	0.0014(0.0586)	0.9990	0.57	42.83	142.74
12	80 – 120	0.0102(0.0020)	0.0020(0.0378)	0.9992	0.85	13.40	44.65
13	40 – 480	0.0324(0.0052)	0.0017(0.0597)	0.9996	1.20	9.11	30.38
14	40 – 360	0.0486(0.0081)	0.0023(0.0540)	0.9993	1.17	9.39	31.30
15	4 – 60	0.0420(0.0022)	0.0106(0.0055)	0.9990	5.19	2.54	8.45

a. Linear range, *n* = 6; t-calc. = t-test of the intercept, calculated value for *t* = 3.182; *s*²*b* = variance of the slope; LOD = limit of detection; LOQ = limit of quantitation.

Table 5 Analysis of cephalosporin dosage forms using proposed and official methods

Dosage forms ^a	Claimed/ mg	% Found $\pm$ SD ^b		t-value ^c	F-value ^c
		Proposed	Official ^d		
Fortum [®] vials	250	99.39 $\pm$ 0.57	98.69 $\pm$ 0.58	1.67	1.04
Cephalexin [®] capsules	250	99.76 $\pm$ 1.28	98.52 $\pm$ 0.73	1.75	3.07
Ceporex [®] tablets	250	99.77 $\pm$ 0.70	99.70 $\pm$ 0.73	0.13	1.09
Clafuran [®] vials	500	96.76 $\pm$ 0.87	98.08 $\pm$ 0.84	2.13	1.07
Cefotax [®] vials	1000	99.13 $\pm$ 1.80	100.75 $\pm$ 1.56	1.34	1.33
Velosef [®] capsules	250	100.00 $\pm$ 0.95	99.82 $\pm$ 1.02	0.25	1.15
Velosef [®] vials	250	— ^e	100.21 $\pm$ 0.86	— ^f	— ^f
Velosef [®] suspension	125	100.50 $\pm$ 1.30	100.53 $\pm$ 1.11	0.03	1.37
Cefzil [®] tablets	250	99.09 $\pm$ 1.16	99.20 $\pm$ 0.80	0.16	2.10
Cefobid [®] vials	500	100.00 $\pm$ 0.82	99.35 $\pm$ 0.56	1.33	2.14
Totacef [®] vials	500	99.47 $\pm$ 1.09	99.01 $\pm$ 0.74	0.71	2.17
Cefatrexyl [®] vials	500	99.00 $\pm$ 0.44	98.56 $\pm$ 0.70	0.98	2.53

a. See Table 2 for details. b. Average of five and three determinations for the proposed and official methods, respectively. c. The tabulated values of *t* and *F* at the 95% confidence limit are 2.37 and 4.88. d. Reference 20. e. High results due to interferences from L-arginine. f. Could not be calculated.

of *p*-CA showed strong bands at 3240, 1661 cm⁻¹, corresponding to OH and C=O stretching, respectively. These bands were shifted by varying the values in the spectra of the complexes.

The investigated cephalosporins have a number of similar functional groups and, thus their IR spectra showed several common absorption bands, *i.e.* in the carbonyl region. All of the compounds showed a β -lactam carbonyl band in a range of 1725 – 1780 cm⁻¹. These carbonyl bands were shifted by a 5 – 40 cm⁻¹ in the spectra of the complexes compared with the spectra of the drugs alone. For example, the β -lactam carbonyl was shifted from 1752 to 1779, 1725 to 1750, and 1740 to 1780 for cefaclor, cefotaxime sodium, and cephaloridine, respectively.

In ¹H-NMR, generally, the protons of the donor are shifted to a lower field (paramagnetic shift). The ¹H-NMR spectra of the complexes of the investigated drugs with *p*-CA were recorded in DMSO-d₆ together with the spectra of the free drugs. In the ¹H-NMR spectra of the complexes of the investigated

cephalosporins, only 6-H and 7-H (but not 2-CH₂) were downfield shifted ($\Delta\delta$ = 0.1 – 0.25 ppm). As reported in the literature^{22,24,25} with other compounds of similar β -lactam antibiotics, it could be preliminary suggested that the sulfur atom did not take part in charge-transfer complex formation, and the lactam nitrogen atom was probably the donor center.

Molecular modeling and computational analysis

Molecular modeling was performed in order to study the effect of the multiple physicochemical parameters of each of the investigated drugs on its reactivity with *p*-CA, and consequently the ultimate sensitivity, expressed as molar absorptivity (ϵ), of the analytical method developed for that particular drug. The geometries of each drug alone, and its complex with *p*-CA were fully optimized at full self-consistent field (SCF) levels^{29,30} by using MOPAC; a general molecular orbital package was implemented with molecular mechanics software MMX-PC.³¹ Each drug-*p*-CA complex was minimized, and the minimization parameters were determined. These parameters were the force

Table 6 Parameters for each of the minimized complexes (drug + chloranilic acid)^a

Drug ^b	ϵ	MMXE	STR	BND	SB	TOR	VDW	DD/QQ	DM	HF	$P/T\%$	Clog P	pK_a	PB	MR
1	2350	5.88	29.46	43.40	-23.19	31.04	-2.64	-72.19	13.07	-185.84	39.60	-3.302	3.63	85	1
2	750	35.76	15.10	36.65	-13.07	19.64	2.03	-24.59	6.36	-251.45	34.89	-1.901	5.00	10.3	1
3	330	50.22	11.71	42.17	-11.59	24.59	0.26	-17.19	8.36	-438.64	33.75	0.565	3.20	25	2
4	920	42.41	13.37	39.48	-12.13	21.96	-2.20	-18.08	11.45	-270.70	49.85	-0.692	5.47	40.5	1
5	2120	35.83	35.33	40.86	-25.71	20.05	6.02	-40.72	9.89	-317.42	51.05	-1.280	3.43	90	2
6	1030	41.33	8.72	36.57	-7.82	24.49	2.80	-23.43	7.15	-286.50	40.65	-2.568	0.26	20	2
7	840	26.11	6.23	37.44	-7.94	19.11	2.80	-31.52	4.49	-138.31	35.90	-1.697	5.58	23.5	4
8	1850	28.48	7.08	38.32	-8.34	14.59	1.64	-24.81	6.14	-247.58	33.82	-1.988	4.90	16.5	1
9	1110	60.93	35.02	44.94	-25.39	24.48	0.95	-19.07	69.6	-269.25	32.60	-0.762	2.35	72.5	2
10	280	47.20	32.48	43.20	-24.82	23.82	-3.05	-24.44	5.04	-385.63	47.32	0.133	2.25	65	2
11	570	44.58	32.62	38.68	-23.99	17.90	4.33	-24.96	6.70	-287.68	36.47	-1.934	6.60	36	2
12	850	12.39	14.03	44.98	-12.69	27.87	4.06	-65.8t7	74.82	-83.18	24.12	-3.642	3.20	27.5	1
13	1200	42.41	35.11	53.77	-25.18	34.87	3.24	-59.39	80.95	-317.13	41.40	0.711	2.60	87.5	1
14	1170	24.14	6.22	51.46	-7.25	29.23	-1.89	-53.62	38.11	-112.26	44.50	-2.081	2.10	78	2
15	5190	113.98	24.99	49.64	-21.62	77.26	0.37	-16.66	20.81	-217.16	41.98	-1.905	0.32	47	2

a. Parameters are: ϵ (molar absorptivity); MMXE (force field); STR (stretching energy); BND (bending energy); SB (stretching: bending); TOR (torsional energy); VDW (Van der Waals forces); DD/QQ (core-core interaction); DM (dipole moment); HF (heat of formation); $P/T\%$ (polar/total surface area); Clog P (calculated log P); pK_a ; PB (protein binding); MR (molar ratio). b. Drug number as shown in Table 1.

Table 7 Correlation matrix for the relation of every minimization parameter with the other parameters

Parameter	ϵ	Clog P	PB	MMXE	STR	BND	SB	TOR	VDW	DD	DP	HF	pK_a	MR	$P/T\%$
ϵ	1.000														
Clog P	-0.289	1.000													
PB	0.215	0.225	1.000												
MMXE	0.565	0.349	-0.018	1.000											
STR	0.174	0.304	0.678	0.268	1.000										
BND	0.344	0.248	0.651	0.256	0.321	1.000									
SB	0.599	-0.532	-0.088	0.265	-0.165	0.095	1.000								
TOR	0.807	-0.020	0.214	0.726	0.210	0.606	0.471	1.000							
VDW	-0.033	-0.130	-0.188	-0.046	0.115	-0.213	0.001	-0.218	1.000						
DD	-0.012	0.371	-0.491	0.637	-0.183	-0.452	-0.006	0.013	-0.048	1.000					
DP	0.046	0.380	0.574	0.178	0.410	0.735	-0.034	0.214	0.019	-0.262	1.000				
HF	0.230	-0.758	-0.051	-0.343	-0.378	0.148	0.628	0.110	0.040	-0.516	0.015	1.000			
pK_a	-0.435	-0.018	-0.277	-0.457	-0.087	-0.502	-0.223	-0.644	0.165	0.027	-0.311	0.061	1.000		
MR	-0.056	0.087	-0.085	0.173	-0.149	-0.176	0.034	-0.005	0.144	0.301	-0.160	0.059	-0.010	1.000	
$P/T\%$	0.192	0.318	0.517	0.194	0.261	0.112	0.128	0.141	-0.298	0.116	-0.036	-0.318	-0.153	0.061	1.000

field (MMXE), stretching energy (STR), bending energy (BND), stretching: bending (SB), torsional energy (TOR), Van der Waals forces (VDW), core-core interaction (DD/QQ), dipole moment (DM), heat of formation (HF), calculated log P (Clog P), pK_a , and protein binding (PB). The polar and total surface area in squared Å² for each drug- p -CA complex were computed to give the parameter of the polar/total surface area ($P/T\%$). The protein binding (PB) and pK_a for each drug were taken from the reported data.³² The calculated log P (Clog P) for each drug was computed by Clog P -PC software.³³ The molar absorptivity (ϵ) and drug: p -CA molar ratio (MR) were determined as described above.

The correlation matrix of each of those parameters with the other parameters was computed (Table 7). A multiple-regression analysis was then performed to derive an equation by which a calculated value for ϵ could be determined. The results are given in Table 8, and the derived equation was:

$$\text{calculated } \epsilon (\pm \text{standard error}) = \text{constant} + \text{sum of multiple parameters values,}$$

where the standard error and the constant in that equation were

75.82 and 5672.77, respectively; the multiple-regression coefficient of this correlation was 0.9999. The validity of this equation was proved by comparing the calculated values of ϵ (Table 6) for each drug with the experimental values (Table 4). The calculated values of ϵ correlated well with those obtained experimentally (Fig. 2). A linear regression analysis of the results yielded the following equation:

$$\text{calculated } \epsilon = 0.3805 + 0.9997 \times \text{experimental } \epsilon.$$

This excellent correlation proved the validity of the proposed computational equation that was derived for calculating the ϵ value for a particular drug based on its physicochemical and geometric parameters; the excellent multiple-regression coefficient (0.9999) indicated the perfect prediction of the ultimate sensitivity of the assay. The proposed equation was then applied for calculating the ϵ value of cefuroxime sodium; the value was 8, indicating a very weak interaction with p -CA, and ultimately very poor assay sensitivity. This was in agreement with our experiments, where we were unable to develop an assay method for that compound by the p -CA reagent.

Table 8 The final equations for calculation of ϵ for any of the related drugs utilizing the constant and the regression coefficient of each parameter^a

Parameter	Regression coefficient	Standard error	Partial r^{2b}
Clog P	-500.53	49.55	0.9903
PB	96.38	3.94	0.9983
MMXE	-226.16	17.49	0.9941
STR	-75.82	6.12	0.9935
SB	32.45	3.84	0.9862
TOR	359.42	21.47	0.9964
VDW	473.90	27.24	0.9967
DD/QQ	244.84	17.31	0.9950
DP	15.22	3.19	0.9979
HF	-13.05	1.07	0.9932
pK_a	437.09	22.81	0.9973
MR	-421.80	43.82	0.9893
$P/T\%$	-123.05	9.53	0.9940

a. See text for multiple parameter equation.

b. Partial r^2 is the correlation coefficient between each parameter and the other parameter in the multiple correlation.

Conclusions

A simple, rapid and accurate spectrophotometric method was developed for the analysis of fifteen cephalosporins via their reaction with p -CA; the reaction was based on charge-transfer complex formation. The proposed procedure could be applied to quality-control analyses of the investigated cephalosporins. The procedure could also be automated; the ongoing work is directed to develop and validate this system. The formation of charge-transfer complexes, and the sites of interaction were confirmed by UV/VIS, IR, 1H -NMR techniques. Molecular modeling for the interaction, and a multiple-regression analysis for the physicochemical and geometric parameters were used to derive an equation for calculating the ϵ value for a particular drug. This equation gave a perfect prediction for the degree of interaction with the p -CA reagent.

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Fig. 2 Correlation of the experimental ϵ with the calculated ϵ (●) and the error % (○). The error % was the difference between the experimental and calculated values, expressed as percentages.