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# Use of charge-transfer complexation in the spectrophotometric analysis of certain cephalosporins

Gamal A. Saleh [a,*], Hassan F. Askal [a], Mohamed F. Radwan [b], Mahmoud A. Omar [c]

[a] *Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt*
[b] *Pharmaceutical Medicinal Chemistry Department, Faculty of Pharmacy, Minia University, Minia, Egypt*
[c] *Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Minia University, Minia 71526, Egypt*

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**Abstract**

Three simple, rapid and sensitive spectrophotometric procedures were developed for the analysis of cephapirin sodium (**1**), cefazoline sodium (**2**), cephalexin monohydrate (**3**), cefadroxil monohydrate (**4**), cefotaxime sodium (**5**), cefoperazone sodium (**6**) and ceftazidime pentahydrate (**7**) in pure form as well as in their pharmaceutical formulations. The methods are based on the reaction of these drugs as n-electron donors with the σ-acceptor iodine, and the π-acceptors: 2,3-dichloro-5,6-dicyano-*p*-benzo-quinone (DDQ) and 7,7,8,8-tetracyanoquinodimethane (TCNQ). Depending on the solvent polarity, different coloured charge-transfer complexes and radicals were developed. Different variables and parameters affecting the reactions were studied and optimized. The obtained charge-transfer complexes were measured at 364 nm for iodine (in 1,2-dichloroethane), 460 nm for DDQ (in methanol) and 843 nm for TCNQ (in acetonitrile). Ultraviolet–visible, infrared and $^1$H-nuclear magnetic resonance techniques were used to study the formed complexes. Due to the rapid development of colours at ambient temperature, the obtained results were used on thin-layer chromatograms for the detection of the investigated drugs. Beer's plots were obeyed in a general concentration range of 6–50, 40–300 and 4–24 μg ml$^{-1}$ with iodine, DDQ and TCNQ, respectively, with correlation coefficients not less than 0.9989. The proposed procedures could be applied successfully to the determination of the investigated drugs in vials, capsules, tablets and suspensions with good recovery; percent ranged from 96.47 ($\pm$1.14) to 98.72 ($\pm$1.02) in the iodine method, 96.35 ($\pm$1.62) to 98.51 ($\pm$1.30) in the DDQ method, and 95.98 ($\pm$0.78) to 98.40 ($\pm$0.87) in the TCNQ method. The association constants and standard free energy changes using Benesi–Hildebrand plots were studied. The binding of cephalosporins to proteins in relation to their molar absorptivities was studied. 

*Keywords:* Spectrophotometry; Charge-transfer complexes; Cephalosporins; Pharmaceutical analysis

* Corresponding author. Tel.: +20-88-411327; Fax: +20-88-332776.
*E-mail address:* gasaleh@acc.aun.eun.eg (G.A. Saleh).

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## 1. Introduction

Reviewing the available colourimetric procedures developed for the analysis of cephalosporins, one can easily recognize that most of these methods invest the cleavage of the β-lactam moiety of cephalosporins structure. These methods include heating with iron(III) and *o*-phenanthroline [1], potassium hexacyanoferrate [2], molybdophosphoric acid [3], chromtrop 2 B and chromtrop 2 R [4], glucitol and sodium hydroxide [5], NBS and NCS [6], copper(II) and vanadium(V) [7], MBTH and 2,6-dichloroquinone-4-chlorimide [8], hydroxylamine perchlorate [9], mercurochrome [10], ferric hydroxamate [11], tetrachloroquinone [12], *p*-benzoquinone and tetrachlorobenzoquinone [13], ammonium molybdate [14], ammonium vanadate [15] and sodium nitroprusside [16]. In addition, we have previously reported the utility of charge-transfer complexation reactions for the analysis of penicillins [17–19] as well as some cephalosporins [20]. The pharmacopoeial methods for the determination of the investigated cephalosporins are either chromatographic methods [21,22] that are expensive or titrimetric methods [22] that are less sensitive. Meanwhile, the other reported methods are lengthy or tedious.

Direct chemical analysis that is based on the reactivity of the intact molecule without its cleavage is not frequently encountered. Therefore, the objective of this paper is to develop a direct analytical procedure that is based on the reactivity of the intact molecule without its cleavage.

## 2. Experimental

### 2.1. Apparatus

A Shimadzu UV 1601, Ultraviolet–visible spectrophotometer (Tokyo, Japan) and Spectronic 21D, UV–Vis spectrophotometer (Tokyo, Japan), both with 1-cm quartz cells, were used. The infrared spectrometer was an IR-470 (Shimadzu, Japan), and the $^1$H-NMR spectrometer was a EM-360, 60 M HZ NMR spectrometer (Varian Instrument Division, Palo, Alto, CA). All calculations were carried out on an IBM computer using the statistical methods in analytical chemistry (SMAC) program, designed by Meier and Zund [23].

### 2.2. Materials and reagents

All solvents used were of analytical-reagent grade. Suppliers were as follows: cephapirin and cefazoline sodium salts (Bristol Myers-Squibb Pharmaceutical Co., Cairo, Egypt), cephalexin monohydrate (Arab Drug Co., Cairo, Egypt), cefadroxil monohydrate (Amoun Pharmaceutical Industries Co., APIC, Cairo, Egypt), cefotaxime sodium and ceftazidime pentahydrate (The Chemical Industries Development Co. [CID], Cairo, Egypt), and cefoperazone sodium (Pfizer Egypt, S. A. E., Cairo, Egypt).

Iodine, resublimed (Riedel-De-Haen AG, Germany), was 25.5 mg/50 ml ($1 \times 10^{-3}$ M) in 1,2-dichloroethane. The solution was found to be stable for at least 1 week at 4°C. 7,7,8,8-Tetracyanoquinodimethane (TCNQ) (Sigma Chemical Co., USA) was 1 mg ml$^{-1}$ in acetonitrile. The solution was found to be stable for at least 1 week at 4°C. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (Sigma Chemical Co., USA) was 2 mg ml$^{-1}$ in methanol. 2,5-Dichloro-3,6-dihydroxy-1,4-benzoquinone (chloranilic acid) was from BDH Chemicals (Poole, UK), 2,3,5,6-tetrabromo-1,4-benzoquinone (Bromanil) from Hopkin & Williams Ltd (UK), and picryl chloride from Sigma Chemical Co. (USA). Tetracyanoethylene (TCNE) (Nacalai Tesque, Kyoto, Japan) was 1 mg ml$^{-1}$ in acetonitrile, prepared fresh daily. 2,3,5,6-Tetrachloro-1,4-benzoquinone (Chloranil) was Prolabo (Rhone-Poulenc, France), 2,3,5,6-tetrafluoro-1,4-benzoquinone (Fluoranil) was from Aldrich-Europe (Beerse, Belgium), and 2,4,7-trinitro-9-fluorenon (TNF) was from Fluka (Switzerland). Fluoranil, chloranil, bromanil, chloranilic acid, picryl chloride and TNF, each 5 mg ml$^{-1}$ in acetonitrile, were prepared fresh daily.

The adsorbent was silica gel G254-precoated plates, and the solvent system was *n*-propanol:water:acetic acid (100:30:5).

### 2.3. Pharmaceutical formulations

The following commercial dosage forms were subjected to the analytical procedure. Cefatrexyl® vials (Bristol Myers-Squibb Pharmaceutical Co., Cairo, Egypt), labelled to contain 500 mg cephapirin sodium per vial. Kefzol® and Totacef® vials (Bristol Myers-Squibb Pharmaceutical Co., Cairo, Egypt), labelled to contain 500 mg cefazolin sodium per vial. Keflex® tablets (Kahira Pharmaceutical Co., Cairo, Egypt), labelled to contain 500 mg cephalexin monohydrate per tablet. Duricef® capsules and suspensions (Bristol Myers-Squibb Pharmaceutical Co., Cairo, Egypt), labelled to contain 250 or 500 mg cephadroxil monohydrate per capsule or 5 ml suspension. Clafuran® vials (Hoechst Orient, S. A. E., Cairo, Egypt), labelled to contain 500 mg cefotaxime sodium per vial. Cefobid® vials (Pfizer Egypt, S.A.E., Cairo, Egypt), labelled to contain 500 mg cefoperazone sodium per vial. Ceftazidime® vials (Chemical Industry Development Co. (CID), Cairo, Egypt), labelled to contain 500 mg ceftazidime pentahydrate per vial.

### 2.4. Procedures

#### 2.4.1. Preparation of standard stock solutions

Into a 50-ml calibrated flask, 24–300 mg drug was weighed accurately and dissolved in 2 ml methanol, completed to volume with the same solvent (for DDQ), with 1,2-dichloroethane (for iodine) and with acetonitrile (for TCNQ), and diluted quantitatively to obtain the suitable concentrations.

#### 2.4.2. General analytical procedures

Aliquot volumes of standard stock solutions, containing 40–3000 μg drug was transferred to 10-ml calibrated flasks. One millilitre of the reagent was added and diluted to volume with 1,2-dichloroethane, methanol, or acetonitrile for the iodine, DDQ, and TCNQ procedures, respectively. The absorbances of the resulting solutions were measured at the wavelength of maximum absorption (364, 460 and 843 nm, respectively) after the appropriate times, at $25 \pm 5$°C against reagent blanks treated similarly.

#### 2.4.3. Stoichiometric study

Job's method of continuous variation [24] was employed. Master equimolar solutions of each drug with iodine ($(2.0–3.8) \times 10^{-4}$ M), DDQ ($1.0 \times 10^{-3}$ M), and TCNQ ($(5.1–5.7) \times 10^{-4}$ M) were prepared in 2.0 ml methanol, and completed to volume with the same solvent (for DDQ), with 1,2-dichloroethane (for iodine) and with acetonitrile (for TCNQ). A series of 10-ml portions of master solutions of each drug with the respective acceptor was made up comprising different complementary proportions (0:10, 1:9, …, 9:1, 10:0) in 10-ml calibrated flasks. The absorbance of the resulting solutions were measured at the wavelength of maximum absorption (364, 460 and 843 nm, respectively) after the appropriate time (20 min for the iodine and DDQ methods, and 45 min in the case of TCNQ) at $25 \pm 5$°C against reagent blanks treated similarly.

#### 2.4.4. Analysis of tablets and capsules

The contents of 20 tablets or capsules of each drug were weighed and powdered or evacuated. A quantity of the powder equivalent to 100 mg was transferred into a 50-ml calibrated flask, dissolved in 2 ml methanol, swirled and sonicated for 2 min, completed to volume with the corresponding solvent (as in standard stock solutions), shaken well for 15 min and filtered, rejecting the first portion of the filtrate and then proceeding as in the general procedure.

#### 2.4.5. Analysis of vials and suspension

A quantity of the powder equivalent to 100 mg was transferred into a 50-ml calibrated flask and dissolved in 2 ml methanol, then proceeding as in the analysis of tablets and capsules.

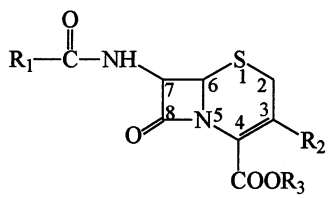
#### 2.4.6. Preparation of the complexes for infrared measurements

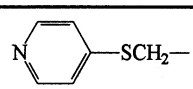
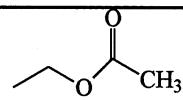
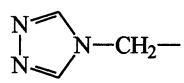
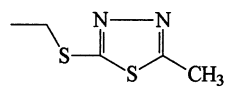
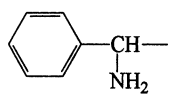
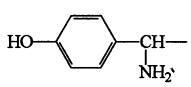
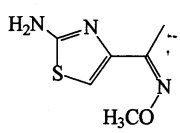
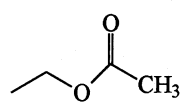
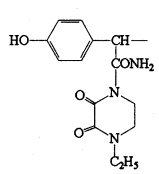
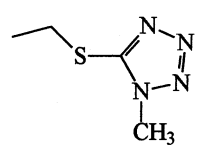
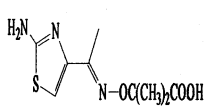
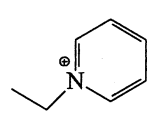
To 2 ml of 0.05 M drug in methanol, 2 ml of 0.05 M of each acceptor in the appropriate solvent (methanol for DDQ, 1,2-dichloroethane for iodine, and acetonitrile for TCNQ) was added in a round-bottom flask containing $\sim 30$ ml appropriate solvent and stirred for 30 min. The solvent was evaporated under reduced pressure and the resulting oily residues were dried over calcium chloride.

*2.4.7. Solutions for $^1H$-nuclear magnetic resonance measurements*

Thirty milligrams of each drug (Table 1) were dissolved in 1 ml $d_6$-DMSO. One millilitre containing an equimolar amount of the acceptors in the same solvent was added and used directly for $^1$H-nuclear magnetic resonance ($^1$H-NMR) measurements.

Table 1
The chemical structure and the percentage of protein binding of the investigated cephalosporins



| Drugs | $R_1$ | $R_2$ | $R_3$ | % protein binding* |
|---|---|---|---|---|
| 1- Cephapirin sodium |  |  | Na | 47 |
| 2- Cefazoline sodium |  |  | Na | 78 |
| 3-Cephalexin monohydrate |  | —$CH_3$ | H | 10 |
| 4-Cefadroxil monohydrate |  | —$CH_3$ | H | 20 |
| 5- Cefotaxime |  |  | Na | 40.5 |
| 6-Cefoperazone sodium |  |  | Na | 87.5 |
| 7-Ceftazidime pentahydrate |  |  | H | 85 |

* Reference 30

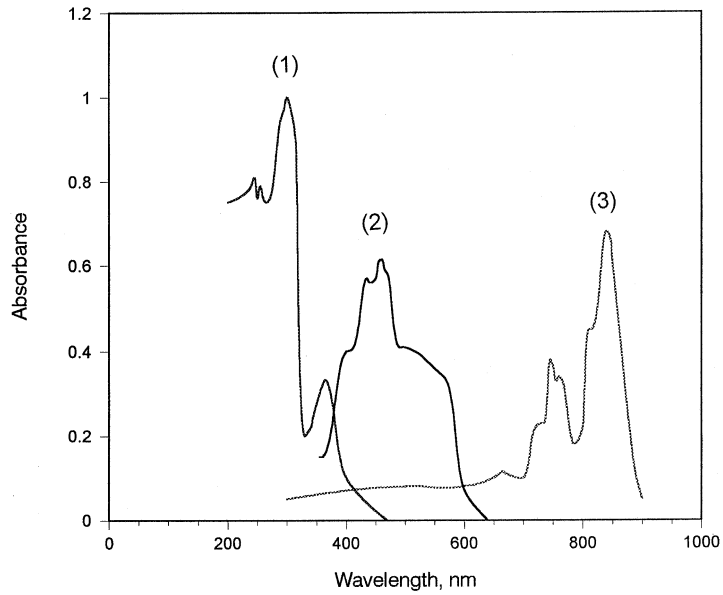



Fig. 1. Absorption spectra of the reaction products of cephapifin sodium (15.75, 130 and 13 μg ml$^{-1}$) with each of iodine (1), DDQ (2) and TCNQ (3), respectively.

## 3. Results and discussion

### 3.1. Reaction with iodine

The violet colour of iodine in 1,2-dichloroethane ($\lambda_{max}$ at 240 and 515 nm) immediately changed into lemon yellow ($\lambda_{max}$ at 290 and 364 nm) on the addition of any of the investigated compounds, dissolved in the smallest amount of methanol and completed with 1,2-dichloroethane, as is typical for charge-transfer complexes (Fig. 1).

The high intensity of the charge-transfer bands is common to complexes of n-donors with iodine [25]. The appearance of absorption peaks at 290 and 364 nm was attributed to the formation of a charge-transfer complex between the investigated drugs and iodine, having an ionized structure $DI^+ \ldots I_3^-$, taking into account that the spectrum of $I_3^-$ in 1,2-dichloroethane shows two absorption maxima at 290 and 364 nm ($\varepsilon = 45\,800$ and $25\,000$ dm$^3$ mol$^{-1}$ cm$^{-1}$, respectively). This complex should originate from an early intermediate outer complex $D \ldots I_2$.

$$D + I_2 \rightleftharpoons \underset{\text{outer complex}}{D - I^+I^-} \rightleftharpoons \underset{\text{inner complex}}{[D - I^+]^-} + I^- \overset{+I_2}{\rightleftharpoons} \underset{\text{tri-iodide ion pair}}{I_3^-}$$

Measurements were carried out at 364 nm due to the interference from the native UV absorption of the studied cephalosporins at 290 nm. The different variables were studied and optimized.

1,2-Dichloroethane was found to be an ideal solvent for the formation of a tri-iodide ion pair (inner complex). Methylene chloride, chloroform and carbon tetrachloride produced lower absorbance readings. Polar solvents were found to be unsuitable as their blanks with iodine gave high absorbances.

The regression equations were derived using the least-squares method [26].

### 3.2. Reaction with π-acceptors

The interaction of any of the investigated compounds with polyhalo- and polycyanoquinone π-acceptors in non-polar solvents (such as dichloroethane) (Table 2) was found to produce coloured charge-transfer complexes with low molar absorptivity values. In polar solvents, such as

acetonitrile or methanol, complete electron transfer from the donor to the acceptor moiety takes place with the formation of intensely coloured radical ions with high molar absorptivity values, according to the following scheme:

$$D + A \rightleftharpoons \underset{\text{complex}}{(D-A)} \overset{\text{polar solvent}}{\rightleftharpoons} \underset{\text{radical ions}}{D^{\cdot +} + A^{\cdot -}}$$

The dissociation of the (D–A) complex is promoted by the high ionizing power of the acetonitrile, and the resulting bands of the named drugs with π-acceptors are similar to the maxima of radical anions of the acceptors obtained by the iodide reduction method [27]. Acetonitrile was considered an ideal solvent as it afforded maximum sensitivity, due to its high dielectric constant that promotes maximum yield of radical anions in addition to its high solvating power of the reagents. Methanol gave maximum sensitivity in the case of DDQ.

The relative sensitivity of the ten acceptors in the analytical work may be compared by their $\varepsilon$ values (Table 2). TCNQ, DDQ and iodine, exhibiting the highest $\varepsilon$ values related to their high electron affinities, were selected for further quantitative work. The weak and small values in cases of picryl chloride, chloranilic acid, chloranil, bromanil, fluoranil, TCNE and TNF may be explained on the basis of insufficient ionization of these relatively weak π-acceptors that possess lower electron affinities [25].

The interaction of all studied drugs with DDQ in methanol at room temperature gave a red-coloured chromogen with a strong absorption maximum at 460 nm (Fig. 1). Different variables were studied and optimized. The interaction of all studied drugs with TCNQ in acetonitrile gave a bluish-green-coloured chromogen with a strong absorption maximum at 843 nm (Fig. 1).

### 3.3. Stoichiometry of the reaction

Using Job's method of continuous variation [24], the molar ratio of either iodine, DDQ or TCNQ to each of the tested drugs was 1:1.

### 3.4. Reagent concentration

The results of reagent concentration variation indicated that 1 ml of either 0.05% iodine, 0.4% DDQ or 0.1% TCNQ is suitable. The higher concentrations used of the reagents may be useful for rapidly reaching equilibrium, thus minimizing the time required to attain maximum absorbance readings at the corresponding maxima.

Table 2
Reaction time, peak position and intensity in polar and non-polar solvents

| Acceptor | Reaction time (min) | 1,2-Dichloroethane[a] | | Acetonitrile[a] | |
|---|---|---|---|---|---|
| | | $\lambda_{max}$ (nm) | $\varepsilon_{max}$ | $\lambda_{max}$ (nm) | $\varepsilon_{max}$ |
| Iodine | 20 | 364 | 6480 | –[b] | –[b] |
| TCNQ | 45 | 605 | 3500 | 842 | 31 800 |
| DDQ | 20 | 388 | 2390 | 460[c] | 21 000 |
| TCNE | 15 | 420 | 735 | 414 | 2740 |
| Fluoranil | 5 | 312 | 2115 | 400 | 3558 |
| Chloranil | 5 | 440 | 1252 | 430 | 2125 |
| Bromanil | 10 | –[d] | –[d] | 425 | 552 |
| Chloranilic acid | 5 | 435 | 412 | 525 | 1073 |
| Picryl chloride | 20 | –[d] | –[d] | 407 | 423 |
| TNF | 15 | 420 | 625 | 414 | 5840 |

[a] Using cefazoline sodium as donor.
[b] High blank readings.
[c] The same readings were obtained with methanol.
[d] No reaction occurred.

Table 3
Association constants ($k_c^{AD}$), correlation coefficients and standard free energy changes ($\Delta G°$) of cephalosporin–iodine (at 364 nm) and cephalosporin–DDQ (at 460 nm) complexes obtained from Benesi–Hildebrand plots

| Drugs | Acceptors | $\Delta G°$ (kJ mol$^{-1}$) | $k_c^{AD} \times 10^3$ (l mol$^{-1}$) | Correlation coefficient[a] ($r$) |
|---|---|---|---|---|
| Cephapirin sodium | $I_2$ | −5.1 | 5.9 | 0.9993 |
| | DDQ | −4.1 | 1.1 | 0.9989 |
| Cefazolin sodium | $I_2$ | −5.3 | 7.6 | 0.9990 |
| | DDQ | −4.2 | 1.2 | 0.9989 |
| Cephalexin monohydrate | $I_2$ | −4.9 | 4.0 | 0.9999 |
| | DDQ | −4.1 | 0.99 | 0.9996 |
| Cefadroxil monohydrate | $I_2$ | −4.9 | 3.7 | 0.9993 |
| | DDQ | −4.1 | 0.99 | 0.9996 |
| Cefotaxime sodium | $I_2$ | −5.2 | 6.6 | 0.9997 |
| | DDQ | −4.1 | 1.1 | 0.9990 |
| Cefoperazone sodium | $I_2$ | −5.0 | 5.2 | 0.9990 |
| | DDQ | −4.2 | 1.2 | 0.9991 |
| Ceftazidime pentahydrate | $I_2$ | −5.1 | 5.2 | 0.9995 |
| | DDQ | −4.1 | 1.1 | 0.9998 |

[a] Average of three detrminations.

### 3.5. Reaction time

The optimum reaction time was determined by following the colour development at ambient temperature ($25 \pm 5$°C). Complete colour development was attained instantaneously or after 5–45 min with all compounds investigated (Table 2), and the colours remain stable for at least a further 10–30 min.

### 3.6. Association constant and free energy change

The association constant for the interaction of each drug with either iodine or DDQ was calculated using the Benesi–Hildebrand equation [28].

$$\frac{[A_o]}{A^{AD}} = \frac{1}{\varepsilon^{AD}} + \frac{1}{K_c^{AD}\varepsilon^{AD}} \times \frac{1}{[D_o]}$$

where $[A_o]$ and, $[D_o]$ are the concentrations of the acceptor and donor respectively, $A^{AD}$ is the absorbance of the complex, $\in^{AD}$ is the molar absorbitivity of the complex, and $K_C^{AD}$ is the association constant of the complex (l mol$^{-1}$ mol).

From the previous equation, on plotting the values of $[A_o]/A^{AD}$ versus $1/[D_o]$, straight lines were obtained, from which the association constants and correlation coefficients were obtained (Table 3). The standard free energy changes of complexation ($\Delta G°$) were calculated from the association constants by the following equation [29].

$$\Delta G° = -2.303RT \log K_c$$

where $\Delta G°$ is the free energy change of the complex (kJ mol$^{-1}$), $R$ the gas constant (1.987 cal mol$^{-1}$ deg$^{-1}$), $T$ the temperature in Kelvin ($273 + °C$), and $K_c$ is the association constant of drug–acceptor complexes (l mol$^{-1}$).

The high values of association constants are common in n-electron donors where the intermolecular overlap may be considerable [24].

### 3.7. Quantification

Under the specified reaction conditions, the molar absorptivity at $\lambda_{max}$ was found to be a function of concentration of the investigated drugs. In all cases studied, Beer's law plots ($n = 6$) were linear with very small intercepts (0.000327–0.02). Slopes ranged from 0.0025 to 0.067 in the concentration ranges presented in Table 4. The regression equations for the three proposed procedures were derived using the least-squares method [25] (Table 4), and the correlation coefficients ranged from 0.9989 to 0.9999.

The mean of eight replicate analyses of solutions of the studied cephalosporins at the concentration limits presented in Table 4 gave relative standard deviation values up to 1.8%. This level of precision of the proposed methods is adequate for the quality control analysis of the studied drugs.

### 3.8. Specificity and interference

The proposed procedures have the advantage that most of the assays are performed in the visible region away from the UV-absorbing interferents that might be co-extracted from dosage forms.

### 3.9. Analysis of pharmaceutical formulations

The obtained high-intensity absorption bands and the very low reagent background make these procedures suitable for the routine quality control analysis of the investigated compounds with minimum interference. The proposed and official methods [21,22] were applied to the determination of the studied drugs in tablets, capsules, suspensions and vials containing different cephalosporins (Table 5). The obtained mean values ($\pm$ S.D.) of the labelled amounts ranged from $96.47 \pm 1.14$ to $98.72 \pm 1.02$ and from $96.36 \pm 1.62$ to $98.51 \pm 1.30$ for iodine, and DDQ and TCNQ, respectively. In the *t*- and *F*-tests, no significant differ-

Table 4
Quantitative parameters for the reaction of the studied cephalosporins with iodine (I), DDQ (II) and TCNQ (III)

| Drugs | | Linear range ($\mu g\ ml^{-1}$) | Intercept (a) | Slope (b) | Correlation coefficient (*r*) | $\varepsilon \times 10^3$ ($dm^3\ mol^{-1}\ cm^{-1}$) | LOD | LOQ |
|---|---|---|---|---|---|---|---|---|
| Cephapirin sodium | (I) | 6–40 | 0.0004 | 0.0222 | 0.9998 | 9.90 | 0.71 | 1.39 |
| | (II) | 40–160 | −0.0055 | 0.0048 | 0.9989 | 2.12 | 4.01 | 7.90 |
| | (III) | 4–16 | −0.0067 | 0.0520 | 0.9999 | 2.29 | 0.09 | 0.17 |
| Cefazoline sodium | (I) | 6–50 | −0.0011 | 0.0137 | 0.9998 | 6.48 | 0.55 | 1.09 |
| | (II) | 40–160 | −0.0041 | 0.0047 | 0.9995 | 2.22 | 2.76 | 5.45 |
| | (III) | 4–12 | −0.0067 | 0.0670 | 0.9997 | 31.90 | 0.23 | 0.45 |
| Cephalexin monohydrate | (I) | 6–40 | 0.0232 | 0.0205 | 0.9992 | 8.31 | 1.27 | 2.49 |
| | (II) | 40–180 | −0.0011 | 0.0046 | 0.9991 | 1.68 | 3.58 | 7.08 |
| | (III) | 6–18 | 0.0034 | 0.0390 | 0.9997 | 14.32 | 0.22 | 0.44 |
| Cefadroxil monohydrate | (I) | 6–40 | 0.0379 | 0.0214 | 0.9991 | 9.64 | 1.37 | 2.68 |
| | (II) | 40–160 | −0.0072 | 0.0048 | 0.9994 | 1.80 | 3.01 | 5.96 |
| | (III) | 4–18 | 0.0100 | 0.0450 | 0.9996 | 16.84 | 0.24 | 0.48 |
| Cefotaxime sodium | (I) | 6–40 | −0.0067 | 0.0184 | 0.9992 | 8.47 | 0.89 | 1.76 |
| | (II) | 40–200 | −0.0011 | 0.0043 | 0.9998 | 1.92 | 1.52 | 3.04 |
| | (III) | 6–18 | 0.0035 | 0.0410 | 0.9998 | 19.77 | 0.13 | 0.34 |
| Cefoperazone sodium | (I) | 6–50 | −0.0010 | 0.0158 | 0.9998 | 10.48 | 0.79 | 1.57 |
| | (II) | 50–180 | 0.0014 | 0.0044 | 0.9994 | 2.94 | 3.44 | 6.79 |
| | (III) | 6–24 | 0.0053 | 0.0330 | 0.9997 | 22.36 | 0.23 | 0.46 |
| Ceftazidime pentahydrate | (I) | 10–40 | 0.0097 | 0.0200 | 0.9989 | 13.35 | 2.01 | 3.91 |
| | (II) | 80–300 | 0.0014 | 0.0025 | 0.9996 | 1.60 | 3.96 | 7.89 |
| | (III) | 6–22 | 0.00033 | 0.0360 | 0.9996 | 23.07 | 0.27 | 0.54 |

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

| Dosage forms | % Found ± S.D.[a] | | | |
|---|---|---|---|---|
| | Iodine | DDQ | TCNQ | Official [21] |
| Cefatrexyl® vials | 97.58 ± 1.04 (*t*, 1.79; *F*, 3.84) | 97.66 ± 1.09 (*t*, 1.61; *F*, 2.42) | 97.83 ± 1.12 (*t*, 1.29; *F*, 2.56) | 98.56 ± 0.70 |
| Totacef® vials | 98.32 ± 1.45 (*t*, 1.03; *F*, 3.32) | 98.51 ± 1.30 (*t*, 0.80; *F*, 3.09) | 97.88 ± 1.51 (*t*, 1.65; *F*, 4.16) | 99.01 ± 0.74 |
| Keflex® tablets | 97.42 ± 1.32 (*t*, 1.75; *F*, 3.27) | 97.81 ± 0.85 (*t*, 1.37; *F*, 1.36) | 97.65 ± 1.39 (*t*, 1.34; *F*, 3.63) | 98.52 ± 0.73 |
| Duricef® capsules | 97.13 ± 1.62 (*t*, 1.41; *F*, 4.21) | 97.77 ± 1.39 (*t*, 1.58; *F*, 3.10) | 96.99 ± 1.87 (*t*, 1.46; *F*, 5.60) | 98.16 ± 0.79 |
| Duricef® suspension | 96.47 ± 1.14 (*t*, 1.10; *F*, 1.64) | 96.35 ± 1.62 (*t*, 1.09; *F*, 3.31) | 95.98 ± 0.78 (*t*, 2.07; *F*, 1.30) | 97.19 ± 0.89 |
| Clafuran® vials | 97.11 ± 1.05 (*t*, 1.59; *F*, 1.74) | 96.92 ± 1.05 (*t*, 2.02; *F*, 1.07) | 96.91 ± 1.02 (*t*, 1.90; *F*, 1.65) | 98.08 ± 0.84 |
| Cefobid® vials | 98.72 ± 1.02 (*t*, 1.30; *F*, 1.56) | 98.32 ± 0.93 (*t*, 2.23; *F*, 2.76) | 98.40 ± 0.87 (*t*, 2.13; *F*, 2.41) | 99.35 ± 0.56 |
| Ceftazidime® vials | 98.21 ± 0.96 (*t*, 1.57; *F*, 2.74) | 97.83 ± 1.23 (*t*, 2.06; *F*, 4.50) | 97.97 ± 1.08 (*t*, 1.95; *F*, 3.47) | 98.69 ± 0.58 |

[a] Three and eight determinations were used for the official and the proposed methods, respectively. The tabulated values of *t* and *F* at the 95% confidence limit are $t = 2.26$ and $F = 19.4$.

ences were found between the calculated and theoretical values (95% confidence) of both the proposed and official methods (Table 5). This indicates similar precision and accuracy.

### 3.10. Identification on thin-layer chromatograms

The different colours developed from the interaction of the investigated drugs with the different acceptors could be used on thin-layer chromatograms for detection and differentiation of these compounds (from their corresponding $R_f$ values: 0.31, 0.32, 0.88, 0.41, 0.51, 0.31, 0.70 for drugs **1**–**7**, respectively). Therefore, spraying with different acceptors revealed the colouration of the spots as yellow (iodine and TCNE), orange–yellow (bromanil), red (DDQ), brown (picryl chloride and TNF), violet (fluoranil, chloranil and chloranilic acid) and bluish-green (TCNQ). In general, the order of decreasing sensitivity is iodine, TCNQ, DDQ, TCNE, fluoranil, chloranil, bromanil, chloranilic acid, picryl chloride and TNF. The rapid development of colours at room temperature with non-corrosive reagents, the variation of colour shades, the sensitivity and the stability of colours suggest obvious use of these acceptor reagents to supplement existing methods for the detection of cephalosporins on chromatograms. The quantitative determination of cephalosporins on thin-layer chromatography using these acceptors is currently investigated.

### 3.11. Investigations on the structure of the charge-transfer complexes (CTC)

The presence of bands at 290 and 364 nm (with iodine), 460 nm (with DDQ) and 843 nm (with TCNQ) indicated the possible CTC formation of the type n–σ or n–π complexes. The formation of such complexes was also confirmed by both IR and $^1$H-NMR measurements.

The majority of infrared measurements on such complexes have been concerned with the shifts in the vibrational frequencies of donors or acceptors (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 [25]. The infrared 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 [25].

The IR spectra of iodine complexes are identical with the spectra of the pure components (iodine is IR inactive). The IR spectra of TCNQ shows strong bands at 2220, 1534 and 800 $cm^{-1}$ corresponding to C≡N, aromatic C=C and 1,4-disubstituted benzene stretching, respectively. These bands were shifted in the spectra of the complexes with the investigated compounds to 2180, 2160, 2140, 1570, 1542 and 853 $cm^{-1}$.

The IR spectra of DDQ shows strong bands at 2220 and 1675 $cm^{-1}$ corresponding to C≡N and C=O of quinone stretching, respectively. The band at 2220 $cm^{-1}$ was shifted to 2200 and 2205 $cm^{-1}$. The band at 1675 $cm^{-1}$ overlaps with the band at 1640 $cm^{-1}$, which corresponds to the C=O stretching frequency.

In $^1$H-NMR, generally, the protons of the donor are shifted to a lower field (paramagnetic shift). The $^1$H-NMR spectra of the complexes of the investigated drugs with different acceptors were recorded in $d_6$-DMSO together with the spectra of the free drugs.

In the $^1$H-NMR spectra of the complexes of cephapirin, cefazolin and cefoperazone (Table 6), since only 6-H and 7-H are downfield shifted ($\Delta\delta = 0.1–0.13$ p.p.m.) and 2-$CH_2$ are not affected, and as reported in the literature [17,19] with similar compounds of related β-lactam antibiotics, it can be preliminary suggested that the sulphur atom is not taking part in the CTC formation and that the lactam nitrogen atom is probably the donor centre.

In the $^1$H-NMR of the complexes of cefadroxil, cefotaxime, ceftazidime and cephalexin, $NH_2$ protons are downfield shifted in addition to the shifts in 6-H and 7-H ($\Delta\delta = 0.1–0.4$ p.p.m.), indicating the participation of the side chain $NH_2$ in the charge-transfer process. In this case, the acceptor molecule will be in the form of sandwich between the two centres of the donor, and a complex of the type $D_2–A_2$ may be formed.

### *3.12. Correlation between molar absorptivities and protein binding*

The binding of cephalosporins to protein contained in the body [30] can influence their action in a number of ways. Therefore, we have studied the possible role of electron donor–acceptor complexes in drug-protein binding using iodine, DDQ and TCNQ as model electron acceptors. The sensitivity of the TCNQ procedure, expressed as log $\varepsilon$ values, varied with different cephalosporins in a systematic fashion that was found to be dependent on the average percent protein binding of the different studied cephalosporins (Table 1). The regression equation for the correlation between log $\varepsilon$ and percentage of protein binding, derived by the least-squares method, was:

Table 6
$^1$H-NMR spectra ($d_6$-DMSO) of cephapirin sodium (**1**), cefazoline sodium (**2**), cephalexin monohydrate (**3**), cefadroxil monohydrate (**4**) cefotaxime sodium (**5**), cefoperazone sodium (**6**) and ceftazidime pentahydrate (**7**), their iodine (**1**–**7a**), DDQ (**1**–**7b**) and TCNQ (**1**–**7c**) preparations

| Compounds | 2-$CH_2$ | 6-H | 7-H | NHCO | $NH_2$ |
|---|---|---|---|---|---|
| **1** | 3.33 | 5.10 | 5.58 | 9.27 | |
| **1a** | 3.33 | 5.20 | 5.58 | 9.27 | |
| **1b** | 3.33 | 5.20 | 5.58 | 9.27 | |
| **1c** | 3.33 | 5.20 | 5.58 | 9.27 | |
| **2** | 3.43 | 5.07 | 5.47 | 9.60 | |
| **2a** | 3.43 | 5.17 | 5.47 | 9.60 | |
| **2b** | 3.43 | 5.22 | 5.47 | 9.60 | |
| **2c** | 3.43 | 5.17 | 5.47 | 9.60 | |
| **3** | 3.40 | 4.95 | 5.63 | –[a] | 8.45 |
| **3a** | 3.40 | 5.05 | 5.70 | –[a] | 8.72 |
| **3b** | 3.40 | 5.05 | 5.70 | –[a] | 8.72 |
| **3c** | 3.40 | 5.05 | 5.70 | –[a] | 8.72 |
| **4** | 2.10 | 4.95 | 5.50 | 9.47 | 4.67 |
| **4a** | 2.03 | 5.05 | –[a] | 9.55 | 5.50 |
| **4b** | 2.00 | 5.35 | 5.75 | 9.55 | 4.84 |
| **4c** | 2.00 | –[a] | 5.75 | 9.55 | 4.84 |
| **5** | 3.30 | 5.15 | 5.60 | 9.47 | 7.20 |
| **5a** | 3.30 | 5.28 | 5.70 | 9.47 | 7.13 |
| **5b** | 3.30 | 5.25 | 5.70 | 9.54 | 7.13 |
| **5c** | 3.36 | 5.25 | 5.70 | 9.54 | 7.13 |
| **6** | 3.40 | 4.90 | 5.50 | 9.30 | |
| **6a** | 3.30 | 5.03 | 5.50 | 9.40 | |
| **6b** | 3.30 | 5.03 | 5.50 | 9.40 | |
| **6c** | 3.30 | 5.03 | 5.50 | 9.40 | |
| **7** | 3.07 | 5.10 | 5.43 | 9.17 | 4.40 |
| **7a** | –[a] | 5.15 | 5.43 | 9.40 | 4.60 |
| **7b** | 2.85 | 5.20 | 5.43 | 9.17 | 4.62 |
| **7c** | 3.04 | 5.17 | 5.43 | 9.17 | 4.62 |

[a] The signal is overlapped with other signals

$$\% \text{ protein binding} = 236.5 \log \varepsilon - 969.8$$

$(r = 0.8131,\ n = 7)$

Iodine and DDQ procedures, being less sensitive, give poor correlations ($r = 0.3419$ and 0.5326, respectively).

## 4. Conclusions

In conclusion, the proposed spectrophotometric procedures are simple and time saving. Moreover, they could be applied to the quality-control analysis of the investigated cephalosporins. In addition to ultraviolet–visible spectrophotometry, infrared and $^1$H-NMR spectroscopy could also be used to study the possible site of interaction between the donors and acceptors.

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