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Potentials of *N*-(2, 4-dinitro-1-naphthyl)-*p*-toluenesulphonamide and 2, 4-dinitro-1-naphthol as novel charge transfer acceptors in pharmaceutical analysis of some quinolones and cephalosporins

O. E. Umoh^{1,2*} , O. A. Adegoke² and D. E. Effiong¹

Abstract

Background: Quinolones and cephalosporins are antibiotic agents with activity against Gram-positive and Gram-negative bacteria. They contain chromophores and amine groups which are electron-rich centres capable of donating electrons to electron-deficient compounds. A survey of the literature revealed that 2, 4-dinitro-1-naphthol, a nitroaromatic useful in chemical synthesis, can accept electrons in charge transfer reactions. This work investigates *N*-(2, 4-dinitro-1-naphthyl)-*p*-toluenesulphonamide and 2, 4-dinitro-1-naphthol in the formation of charge transfer complexes by accepting electrons from selected quinolones and cephalosporins. Five other nitroaromatics (i.e. 4-nitro-1-naphthylamine, 2-nitro-1-naphthol, 2,4-dinitro-1-naphthylamine, 1-nitronaphthalene and 1,4-dinitronaphthalene) were screened in addition to the aforementioned and compared for charge transfer complexes formation. Spot test was used to establish charge transfer complex formation at room and elevated temperatures with determination done by visual inspection and thin layer chromatographic analysis of the reaction mixture. Ultraviolet visible absorption spectroscopy was used to estimate the extent of complexes.

Results: Only solutions of adducts of *N*-(2, 4-dinitro-1-naphthyl)-*p*-toluenesulphonamide and 2, 4-dinitro-1-naphthol gave instant and distinct colour with each drug used at room and elevated temperature. While the former gave deep golden yellow, the latter gave golden yellow against their blank reagent solutions which were, lemon and greenish yellow respectively. Visual inspections of 2-nitro-1-naphthol adduct solutions showed no colour change from the yellow colour of the blank reagent solution, even though the Ultraviolet visible absorption spectra revealed the formation of charge transfer complexes. The adducts solutions of 4-nitro-1-naphthylamine, 2,4-dinitro-1-naphthylamine, 1-nitronaphthalene and 1,4-dinitronaphthalene showed no colour change from their blank reagent solutions and their Ultraviolet visible absorption spectra revealed no formation of charge transfer complexes.

Conclusion: Ultraviolet visible absorption spectral analysis shows superiority of *N*-(2, 4-dinitro-1-naphthyl)-*p*-toluenesulphonamide and 2, 4-dinitro-1-naphthol in charge-transfer complex formation over other nitroaromatics screened. *N*-(2, 4-dinitro-1-naphthyl)-*p*-toluenesulphonamide and 2, 4-dinitro-1-naphthol are good acceptors of electrons from

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these drugs, hence could be useful as charge transfer reagents in ultraviolet visible spectrophotometric analysis of these drugs.

Keywords: Nitroaromatics, Quinolones, Cephalosporins, Charge-transfer complex formation

Background

Quinolones and cephalosporins are antibacterial agents active against Gram-positive and Gram-negative bacteria. Quinolones act by inhibiting the bacterial DNA gyrase, truncating DNA replication and transcription [1]. Their activities also cover mycobacteria, mycoplasmas and rickettsias. Significant development in fluoroquinolone research has led to synthesis of new analogues such as norfloxacin, pefloxacin, amifloxacin, ofloxacin, rufloxacin, and enoxacin, more potent and with broader spectra of activity than the parent compound, nalidixic acid. Quinolones have 1, 8-naphthyridine pharmacophore, derived from nalidixic acid. Chemically introducing 6-fluoro and 7-piperazinyl substituents on the primary pharmacophore extends the spectrum of activity, increase potency and overcome plasma-mediated resistance [2]. Several methods for the assay of quinolones in bulk powder, formulations and biological fluids include, titrimetric for nalidixic acid and non-aqueous titration method for ofloxacin, sparfloxacin and gatifloxacin just as chromatographic approach have also been employed [3–8]. A high performance thin layer chromatographic (HPTLC) using a fluorescence based densitometric quantification at 313 nm have been reported for routine analysis of quinolones while spectrofluorimetry using charge transfer formation with 7, 7, 8, 8-tetracyanoquinodimethane acting as π -electron acceptor as well as charge-transfer complex with fluoranil π -electron acceptor gave positive outcome [9–11]. Atomic absorption spectroscopy and spectrophotometric techniques have been reported for fluoroquinolones in bulk formulations and biological matrix [12–17].

Cephalosporins, antibiotic agents from *Cephalosporium acremonium* are indicated in infections of the respiratory tract, urinary tract and in patients with penicillin hypersensitivity. Chemically, related to penicillins with beta-lactam ring structure, cephalosporin possess nucleus, 7-aminocephalosporanic acid (7-ACA), which is analogous to penicillin nucleus, 6-aminopenicillanic acid (6-APA), and is a derivative of acid-stable cephalosporin C. The 7-ACA comprises of dihydrothiazine nucleus; a 6-membered ring containing a double bond unlike the 5-membered ring in penicillin. Several analytical methods have been employed by researchers to assay cephalosporins in formulations and biological samples.

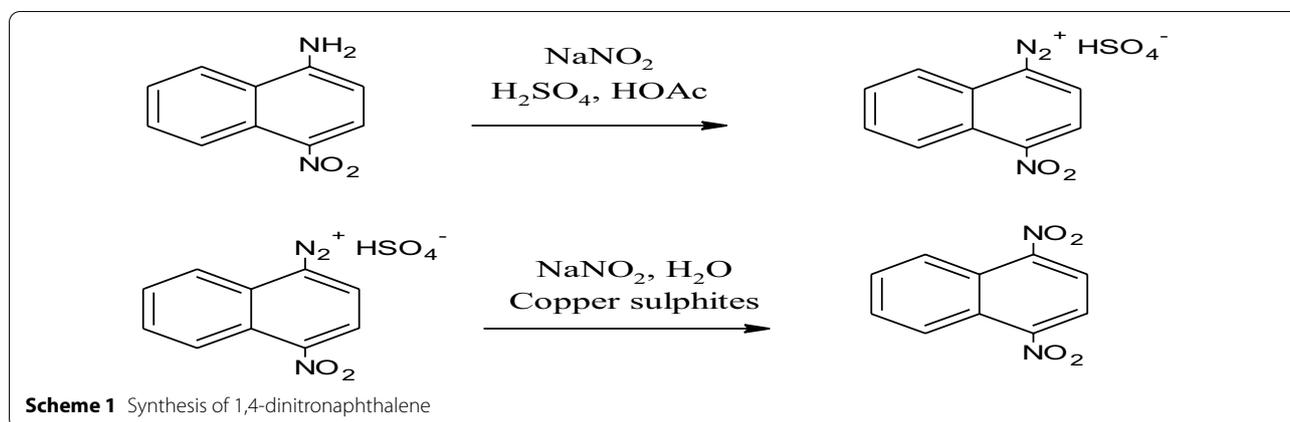
Moreno and Salgado [18] developed and compared acidimetric, iodometric and non-aqueous titrimetric

methods for the assay of ceftazidime in varying drug formulations and investigated infrared spectroscopy for assay of injections of the same drug. Chemiluminescent and atomic absorption spectrometric methods were developed for analysis of cefadroxil monohydrate as well as cefotaxime sodium and cefuroxime sodium, respectively [19, 20]. Spectrophotometric methods for analysis of cephalosporins in bulk powders and formulations have been used, employing molybdophosphoric acid as the oxidant, and diazotisation and coupling with p-dimethylaminobenzaldehyde [21, 22].

Other methods reported are voltammetry and square-wave voltammetry techniques for the adsorption behaviour of cefonicid, cathodic stripping voltammetric technique for Cephapirin, cefamandole and cefmetazole, hanging mercury drop electrode for electro-reduction behaviour and determination of cefoperazone, polarographic methods for cefotaxime and ceftriaxone in drug formulations and isocratic HPLC method for cephalosporins in human serumas well as liquid chromatography [23–29]. Also, Aléssio and Salgado [30] described an agar diffusion method for the determination of ceftriaxone sodium powder for injection based on the inhibition of *Bacillus subtilis*.

Nitroaromatic compounds (NACs) are synthetic compounds useful as synthetic intermediates in production of dyes, pesticides, agrochemicals. They are closely related to chloronitroaromatics (CNACs), precursors for synthesis of drugs, herbicides and dyes [31]. These compounds contain a strong ring deactivating nitro substituent, hence unreactive in electrophilic substitution reactions [32]. This electron withdrawing capacity of the nitro-groups of these compounds which make them electron deficient is tested and used by screening some selected NACs as charge transfer reagents (electron acceptors) with some drug substances possessing excess π - or n -electrons. As at present, the literature survey revealed the use of few nitroaromatic derivatives as charge transfer reagents, and involved 2, 4-dinitro-1-naphthol, besides their use in chemical synthesis [31–34].

Most analytical methods for quinolones and cephalosporins involve multiple steps, elaborate experimental setup, laborious and expensive, elongated time and large sample size and are often of poor sensitivity [5, 35]. Besides, none of these methods employs nitroaromatic derivative as derivatising reagents. The nitroaromatics could therefore be used as derivatising agents for



pharmaceutical analyses. It can also be used as reagents for analytical methods developments for compounds other than pharmaceuticals.

This present work is aimed at screening some nitroaromatic compounds for use as novel analytical agents for simple, rapid single-step assays of quinolones and cephalosporins in bulk dosage forms and in biological fluids and validating their accuracy and precision using spectrophotometric analysis. This will find application in on-field testing without sophisticated instrumentation, on the spot detecting of drug adulteration and counterfeit medicines and in forensic analysis.

Method

Chemicals and reagents

The reagents and solvents used were of analytical grade and include acetone, ethanol, methanol, chloroform, ethyl acetate, 1, 4-dioxan, sulphuric acid, hydrochloric acid, perchloric acid, acetic anhydride, glacial acetic acid, acetonitrile, petroleum ether, diethyl ether (all BDH, England); 1-nitronaphthalene, 2-nitro-1-naphthol, 2, 4-dinitro-1-naphthol, 4-nitro-1-naphthylamine and *N*-(2, 4-dinitro-1-naphthyl)-*p*-toluenesulphonamide (Sigma-Aldrich, USA); 1, 4-dinitronaphthalene and 2, 4-dinitro-1-naphthylamine (synthesised and purified in our laboratory).

Drug samples

Sparfloxacin (SPA), norfloxacin (NOR), ofloxacin (OFL), ceftriaxone injection (CFR), ceftazidime injection (CFT), and cefuroxime injection (CFU) (extracted, crystallised and recrystallised in our laboratory).

Equipment

Digital analytical balance (KERN AL 220-4, Kern and Sohn own DKD Calibration Laboratory, Germany); Mettler analytical balance (H 80, Mettler, UK); UNICO UV-2100 series (Shanghai Instruments Company Ltd; China), Ultrasonic bath (Langford, Ultrasonic sonomatic®; UK); UV lamp (Jenway UV 7804, UK); Silica gel GF254 precoated plates (G. Merc Germany); Vortex mixer (XH-C, Wincom Co. Ltd, China); Stuart melting point apparatus (SMP 11 with thermometer, Stuart Co. Ltd, England); Oven (Techmel AISET YLD-2000, Techmel, USA.), HPLC equipment (Agilent Technologies 1260 Infinity, USA.); UV/VIS spectrophotometer (Spectrum-lab 752s., B. Bran Scientific and instrument company, England) and infrared spectrophotometer (Spectrum Two FT-IR PerkinElmer and PerkinElmer FT-IR BX II, USA).

Synthesis

These nitroaromatics, 1, 4-dinitronaphthalene and 2, 4-dinitro-1-naphthylamine were synthesised via Sandmeyer's reaction from 4-nitro-1-naphthylamine and the hydrolysis of *N*-(2, 4-dinitro-1-naphthyl)-*p*-toluenesulphonamide [*N*-(2, 4-DN1NL) PTS], respectively [36, 37] as shown in Schemes 1 and 2 below.

Procedure and steps for the synthesis of 1, 4-dinitronaphthalene from 4-nitro-1-naphthylamine

Into a 50-mL beaker placed in an ice bath, a 200 mg of powdered sodium nitrite was dissolved in conc. sulphuric acid (1.0 mL). Into another 50-mL beaker in an ice bath, a 200 mg of 4-nitro-1-naphthylamine (4-N1NA) was also dissolved in 1.0 mL conc. H₂SO₄. Maintaining the temperatures of both baths at 5 °C by the use of ice bath, the contents of both beakers were mixed together and the solution well stirred (i.e. solution of 4-N1NA into

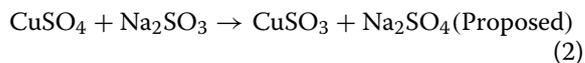
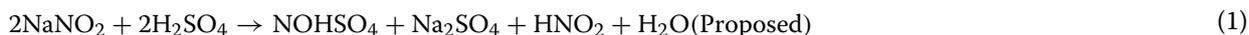
solution of nitrosylsulphuric acid) into glacial acetic acid (5 mL) in a 50-mL beaker, also maintained at 5 °C by the use of ice bath.

The sticky precipitate of 4-nitronaphthalene-1-diazonium sulphate was precipitated with 4–10 mL dry ether after half an hour; and thereafter treated with 5 mL of 95% ethanol after supernatant removal to make it granular. This was washed several times using ether and 95% ethanol successively to remove the acid before eventual dissolution in ice water (2 mL).

Thereafter, saturated aqueous solution (3 mL) of crystalline copper sulphate with crystalline sodium sulphite (1 g each) was prepared as the decomposition mixture. The resulting greenish-brown precipitate was harvested, water-washed and emptied into a solution of sodium nitrite (2 g/8 mL of water) in a beaker. The content was mixed by stirring using a glass rod.

A considerable frosting occurred when the cold aqueous solution of 4-nitronaphthalene-1-diazonium sulphate was added slowly to the decomposition mixture which was defrosted at 10 min interval by addition of five drops of ether. After an hour of stirring, to aid dissolution of the inorganic materials, the mixture was filtered and the crude dark precipitate of 1, 4-dinitronaphthalene was collected, and washed with water, 2% sodium hydroxide and water in successively fashion. This was then dried, extracted with hot 95% ethyl alcohol before the extract was concentrated to 1.5 mL. Most of the 1, 4-dinitronaphthalene that separated was collected and dried at 65°C. Finally, the melting point of the pale yellow crystals that were collected was noted. The ultraviolet and infrared spectra were also used to differentiate between the synthesised and the starting material.

Equations:



Procedure and Steps for the synthesis

of 2, 4- dinitro-naphthylamine

from N-(2,4-dinitro-1Naphthyl)-p-toluenesulphonamide

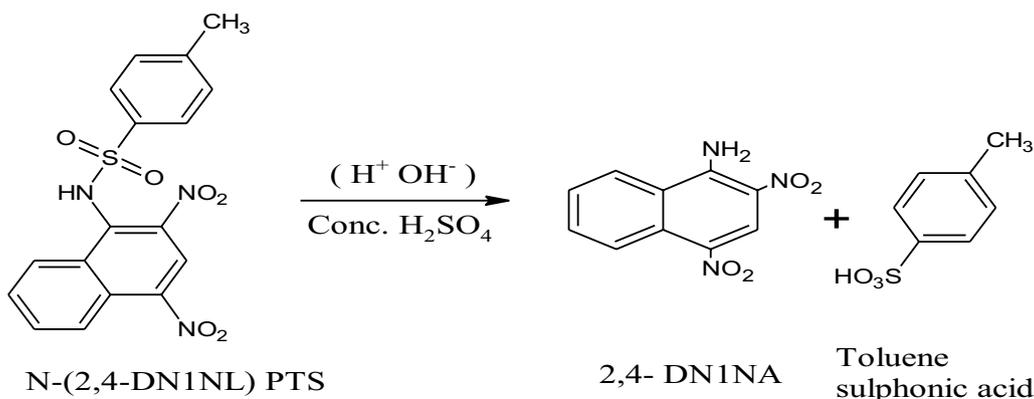
A 300 mg of dried N-(2, 4-dinitro-1-Naphthyl)-p-toluenesulphonamide was added to 0.74 mL of conc. H_2SO_4 containing 0.06 mL of distilled water; and stirred in a beaker maintained at temperature of 20 °C. This solution was gently poured into ice after 45 minutes for the brightly coloured lemon yellow precipitate of 2,4-dinitro-1-Naphthylamine to come out; which was harvested, washed with water and dried at temperature of 65 °C. This was recrystallized and the melting point noted. Ultraviolet, infrared spectroscopy and Hinsberg's tests for amines were also used to differentiate the synthesized reagents from the starting material.

Preparation of stock solutions of 2, 4-dinitro-1-naphthol and N-(2, 4-dinitro-1-naphthyl)-p-toluenesulphonamide

A 1 mg/mL (1000 µg/mL) of these reagents were made by dissolution of 10 mg each of the reagents in acetonitrile in a 10-mL volumetric flask.

Drugs stock solutions

Each drug (10 mg) was dissolved in methanol using a 10-mL volumetric flask. Equimolar concentrations of each of the drugs (0.00427 M) of 2, 4-DN1N and (0.00258 M) of N-(2, 4-DN1NL) PTS were made in methanol as stock solutions.



Scheme 2 Hydrolysis of N-(2, 4-DN1NL) PTS at room temperature

Charge transfer reactions

The stock solution of the drug (0.50 mL) was reacted with the 2, 4-DN1N solution (0.5 mL) inside a test tube, vortex-mixed, and then made up to 5 mL with acetonitrile. The colour change at 30 °C was recorded immediately and after 5 min; and at 60 °C, after 5 min and 20 min. This procedure was repeated with *N*-(2, 4-DN1NL) PTS.

Thin-layer chromatography

Thin-layer chromatographic (TLC) examination was conducted using three different solvent systems, chloroform/ethyl acetate/methanol (4:4:2) for the quinolones and cephalosporins using 2, 4-dinitro-1-naphthol as charge transfer reagent. Pre-coated TLC plates were spotted with the stock solutions (i.e. 0.1% solution of 2, 4-DN1N and solutions of equimolar concentrations of each of the drug candidates) of drug candidates, 2, 4-DN1N, and adducts to show evidence of reaction. The developed plates were air-dried and visualised in daylight and under UV lamp at 254 nm. Spots identified under UV light were marked and the retardation factor (R_f) values calculated. This procedure was repeated with *N*-(2, 4-DN1NL) PTS. Only reaction mixtures of drugs that produced colours at room and/or elevated temperature were considered for TLC analysis.

UV-VIS absorption spectrum determination

The absorption spectrum was done using the studied drugs with the selected nitroaromatics. A 0.10 mL aliquot of 1000 µg/mL of 2, 4-dinitro-1-naphthol was transferred into a clean test tube and 0.10 mL of each drug candidate's stock solution was added. The immediate golden yellow coloured complex formed was allowed to stay at 30 °C for 5 min. The solution was made up to 10 mL with acetonitrile, vortex-mixed and scanned between 200 and 600 nm in a UV-visible spectrophotometer using acetonitrile as the blank. The absorbance readings were recorded at interval of 10 nm. These values were used to generate the respective spectra. These procedures were repeated for all the drug candidates. A 0.10-mL stock solution of 2, 4-DN1N was diluted to 10 mL in a volumetric flask with acetonitrile as the reagent blank, vortex-mixed and scanned from 200 to 600 nm with the use of UV-spectrophotometer to establish the λ_{\max} of the reagent. This procedure was repeated with *N*-(2, 4-DN1NL) PTS.

Preparation of solid charge transfer (CT) complexes

The complexes of charge transfer between the drugs and nitroaromatics were prepared by mixing equimolar amount of drugs and reagents in acetonitrile and then stirred for about 45–60 min. The solid precipitated after a reduction in volume of the solvent due to evaporation

at room temperature. The complexes were separated, filtered, washed with acetonitrile and dried. The melting point and colour were noted.

Results

Charge transfer complex formation was carried out as described between the selected cephalosporins and quinolones (Table 1) and the seven selected nitroaromatic derivatives (Fig. 3). All the drugs studied showed evidence of charge transfer formation with *N*-(2, 4-dinitro-1-naphthyl)-*p*-toluenesulphonamide {*N*-(2, 4-DN1NL) PTS}, 2, 4-dinitro-1-naphthol (2, 4-DN1N) and 2-nitro-1-naphthol (2-N1N) as shown in Figs. 1a–c and 2a–c for cephalosporins and quinolones, respectively. 4-nitro-1-naphthylamine (4-N1NA), 2, 4-dinitro-1-naphthylamine (2, 4-DN1NA), 1-nitronaphthalein (1-NNaph) and 1, 4-dinitonaphthalein (1, 4-DNNaph) did not show any significant evidence of charge transfer complex formation with the studied drugs as shown in Figs. 1d–g and 2d–g for cephalosporins and quinolones, respectively. The results of spot tests including chromatographic R_f values of adducts of *N*-(2, 4-DN1NL) PTS and 2, 4-DN1N are shown in Tables 2 and 3, respectively. The abbreviations, colours and melting points of isolated adducts for *N*-(2, 4-DN1NL) PTS and 2, 4-DN1N are also given in Tables 4 and 5 for a clearer distinction of colours of different adducts formed with cephalosporins and quinolones, respectively.

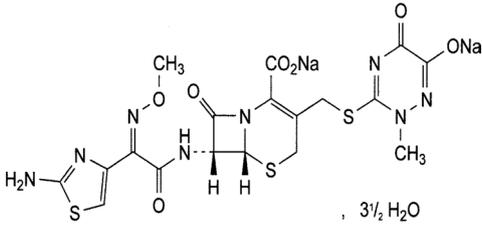
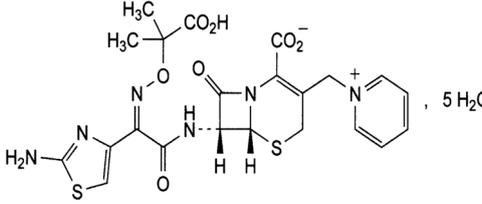
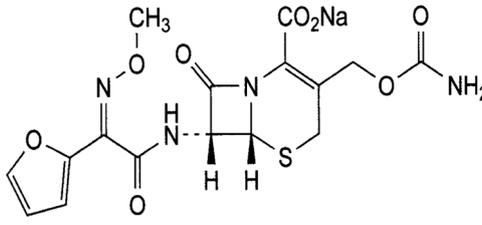
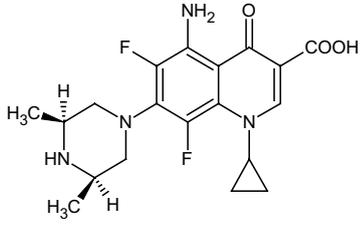
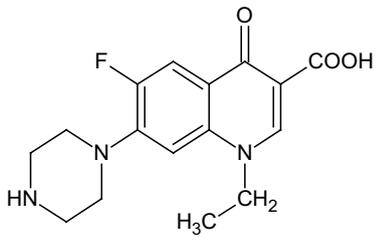
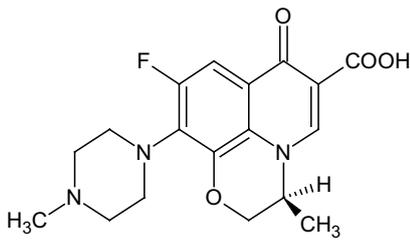
The UV-VIS spectra of screened nitroaromatics (reagents) in Fig. 3 are presented in Figs. 1a to 2g. Their structural differences, observations and deductions are discussed with regards to charge transfer complex formation with the studied drugs. UV-VIS spectra of screened reagents using cephalosporins (CFR, CFT and CFU) as donor molecules are presented in Fig. 1a–g, whereas the spectra for the quinolones are presented in Fig. 2a–g.

Discussion

Three of the seven reagents screened, *N*-(2, 4-DN1NL) PTS, 2, 4-DN1N and 2-N1N showed evidence for charge transfer complex formation with drug candidates; and the UV-VIS spectra of their complexes showed bathochromic shift compared with the spectra of the reagents. The isolated adducts of *N*-(2, 4-DN1NL) PTS and 2, 4-DN1N gave a clearer distinction in colours of the complexes formed when observed as solids.

One significant feature of this screening procedure was the speed with which *N*-(2, 4-DN1NL) PTS and 2, 4-DN1N formed adducts (complexes) with the studied drugs at room and elevated temperatures. These points to the facts that electrons were readily donated by these drugs to these nitroaromatics as evidenced by instantaneous formation of deep golden and golden yellow colours,

Table 1 Chemical structures and pharmacological action of drugs used in this study

S. no	Name and pharmacological action	Chemical structure
1	Ceftriaxone disodium salt (antimicrobial agent with broad spectrum of activity, useful in pelvic inflammatory diseases, systemic sepsis, etc.)	
2	Ceftazidimepentahydrate (antimicrobial agent with broad spectrum of activity, useful in systemic sepsis, etc.)	
3	Cefuroxime sodium salt (antimicrobial agent with broad spectrum of activity, useful in lower respiratory tract infections)	
4	Sparfloxacin (antimicrobial agents with activity against community-acquired pneumonia, and acute exacerbations of chronic bronchitis)	
5	Norfloxacin (antimicrobial agents with activity against urinary tract infections, sexually transmitted diseases, skin and soft tissue infections)	
6	Ofloxacin (antimicrobial agents with activity against urinary tract infections, sexually transmitted diseases, skin and soft tissue infections)	

respectively, as observed in the reaction mixtures. The charge transfer complexes formation with these nitroaromatics presided with a change in the λ_{\max} of reagents,

250 and 210 nm to 440 and 450 nm for adducts of *N*-(2, 4-DN1NL) PTS and 2, 4-DN1N, respectively. The instantaneous formation of colours also suggested that the

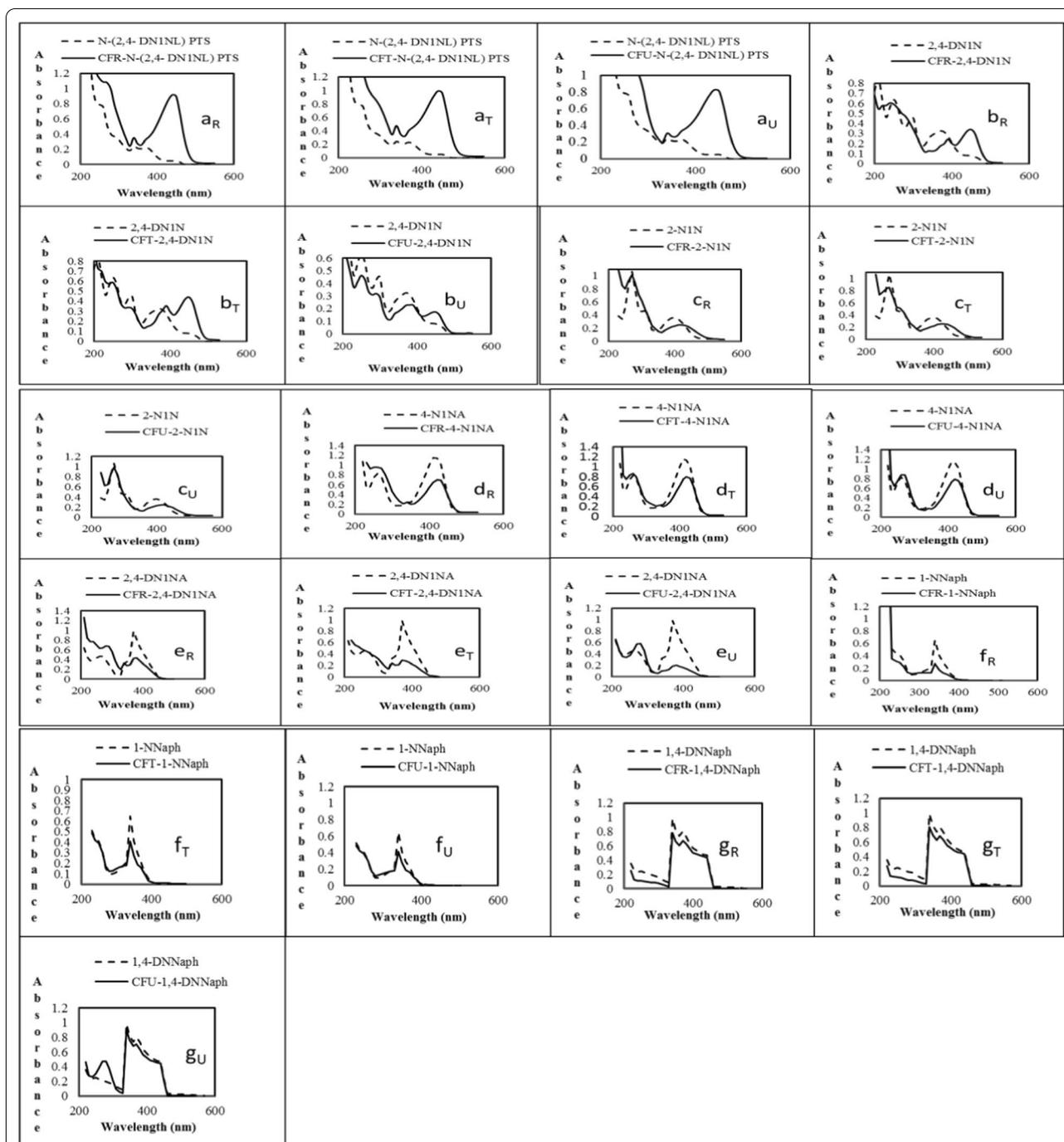


Fig. 1 Overlaid absorption spectra of adducts (—) and reagents (-----), where a-g represents the nitroaromatics screened but the subscript is the specific cephalosporins screened. a = -N-(2, 4-DN1NL) PTS, b = 2, 4-DN1N, c = 2-N1N, d = 4-N1NA, e = 2, 4-DN1NA, f = 1-NNaph, g = 1, 4-DNNaph, _U is cefuroxime, _R is ceftriaxone and _T is ceftazidime

primary, secondary, and tertiary amino groups found in the studied drugs are good electron donors and that the complexes are produced readily. It also implies that these two nitroaromatics are good electron acceptors probably due to the presence of two nitro groups that

withdraws electrons from the rings and the presence of the acidic hydrogen in these molecules. The reaction and formation of charge transfer complexes was different in other nitroaromatics screened, probably due to structural differences in the molecules. In these ones,

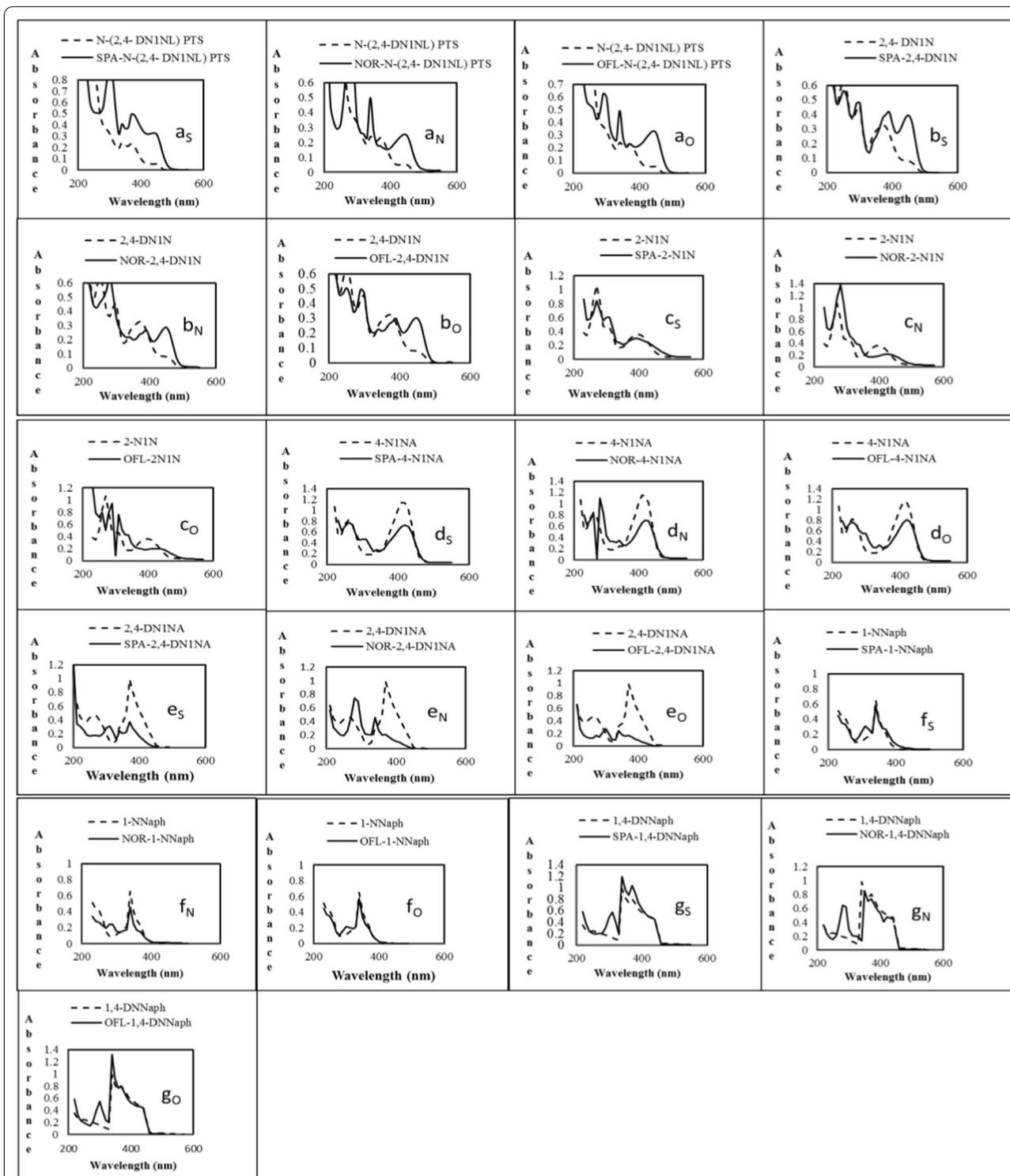


Fig. 2 Overlaid absorption spectra of adducts (—) and reagents (----), where a-g are the screened nitroaromatics but subscripts stand for the specific quinolones used. a = *N*-(2, 4-DN1NL) PTS, b = 2, 4-DN1N, c = 2-N1N, d = 4-N1NA, e = 2, 4-DN1NA, f = 1-NNaph, g = 1, 4-DNNaph. _O is Ofloxacin, _S is sparfloxacin and _N is Norfloxacin

Table 2 Spot test results and chromatographic R_f values for *N*-(2, 4-DN1NL) PTS-azo adduct formed with drugs studied

Drugs	Colour of adducts at 30 °C		Colour of adducts at 60 °C		R_f values ** (CHCl ₃ : EtOAc: MeOH (4:4:2 v/v))
	Instantly	5 min	5 min	20 min	
Sparfloxacin	Deep golden yellow	Deep golden yellow	Deep golden yellow	Deep goldenyellow	0.35 and 0.83 (0.35)
Norfloxacin	Deep golden yellow	Deep golden yellow	Deep golden yellow	Deep golden yellow	0.043 and 0.83 (0.043)
Ofloxacin	Deep golden yellow	Deep golden yellow	Deep golden yellow	Deep golden yellow	0.043 and 0.83 (0.043)
Ceftriaxone	Deep golden yellow	Deep golden yellow	Deep golden yellow	Deep golden yellow	0.014 and 0.83 (0.014)
Ceftazidime	Deep golden yellow	Deep golden yellow	Deep golden yellow	Deep golden yellow	0.014 and 0.83 (0.014)
Cefuroxime	Deep golden yellow	Deep golden yellow	Deep golden yellow	Deep golden yellow	0.014 and 0.83 (0.014)

*Blank reagent solution without drug is lemon yellow; ** R_f of drug substance in parenthesis (R_f of reagent = 0.83)

Table 3 Spot test results and chromatographic R_f values for 2, 4-DN1N-azo adduct formed with drugs studied

Drugs	Colour of adducts at 30 °C		Colour of adducts at 60 °C		R_f values** (CHCl ₃ : EtOAc: MeOH (4:4:2 v/v))
	Instantly	5 min	5 min	20 min	
Sparfloxacin	Golden yellow	Golden yellow	Golden yellow	Golden yellow	0.23 and 0.81 (0.23)
Norfloxacin	Golden yellow	Golden yellow	Golden yellow	Golden yellow	0.10 and 0.81 (0.10)
Ofloxacin	Golden yellow	Golden yellow	Golden yellow	Golden yellow	0.10 and 0.81 (0.10)
Ceftriaxone	Golden yellow	Golden yellow	Golden yellow	Golden yellow	0.13 and 0.81 (0.13)
Ceftazidime	Golden yellow	Golden yellow	Golden yellow	Golden yellow	0.10 and 0.81 (0.10)
Cefuroxime	Golden yellow	Golden yellow	Golden yellow	Golden yellow	0.10 and 0.81 (0.10)

*Blank reagent solution without drug is greenish yellow; ** R_f of drug substance in parenthesis (R_f of reagent = 0.81)

Table 4 Abbreviations, colours and melting points of isolated adduct using *N*-(2, 4-DN1NL) PTS as charge transfer reagent

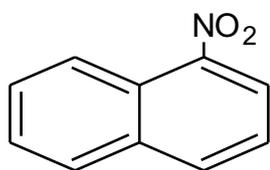
S. no	Abbreviation of adducts	Melting point °C	Colour of adducts
1	SPA-N-(2,4-DN1NL) PTS	168–170	Light golden yellow
2	NOR-N-(2,4-DN1NL) PTS	155–157	Golden yellow
3	OFL-N-(2,4-DN1NL) PTS	201–204	Golden yellow
4	CFR-N-(2,4-DN1NL) PTS	208–210	Golden yellow
5	CFT-N-(2,4-DN1NL) PTS	176–178	Light golden yellow
6	CFU-N-(2,4-DN1NL) PTS	158–160	Pale golden yellow

Table 5 Abbreviations, colours and melting points of isolated adducts using 2, 4-DN1N as charge transfer reagent

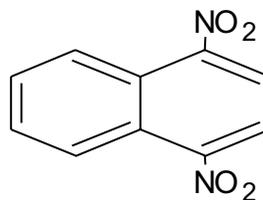
S. no	Abbreviation of adducts	Melting point °C	Colour of adducts
1	SPA-2,4-DN1N	208–211	Light orange
2	NOR-2,4-DN1N	224–226	Golden yellow
3	OFL-2,4-DN1N	218–220	Golden yellow
4	CFR-2,4-DN1N	220–224	Orange
5	CFT-2,4-DN1N	183–185	Bright orange
6	CFU-2,4-DN1N	192–194	Orange

solutions of adducts were not different from their blank reagent solutions as evidenced by spot test. Chromatographic resolutions were able to ascertain the purity of the cephalosporins and quinolones examined, as only one clear spot is shown in the columns of drug samples. The presence of two spots in the columns of the complexes indicates two entities in each of the complexes. The inability of having one spot is presumably due to incorrect solvent system or the presence of unreacted drugs since the droplets were not combined stoichiometrically.

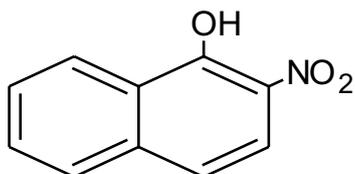
N-(2, 4-DN1NL) PTS has two nitro groups as substituents. Besides $-\text{NO}_2$ groups, the presence of $-\text{SO}_2$, $-\text{NH}$ groups and additional benzene ring lends credence to the formation of charge transfer complexes with the studied drugs. The lone pair of electrons of oxygen atoms of $-\text{SO}_2$ group and that of the nitrogen of amide engaged in resonance arrangement with pi-electrons of aromatic rings. This resonance stabilisation engaged the lone pair of electrons of the nitrogen of amide, and thus, causing the hydrogen ($-\text{NH}$) to be partially acidic thereby taking part in intra-molecular and intermolecular hydrogen bond formation as it is the case with 2, 4-dinitro-1-naphthol [34]. The complexes of *N*-(2, 4-DN1NL) PTS with the studied drugs therefore showed bathochromic shifts as shown in Figs. 1a, and 2a for cephalosporins and



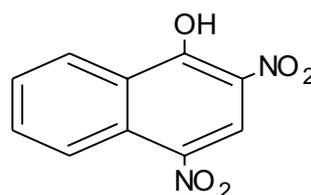
1-nitronaphthalene
(1-NNaph) (I)



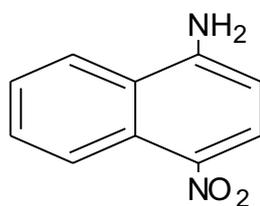
1,4-dinitronaphthalene
(1,4-DNNaph) (II)



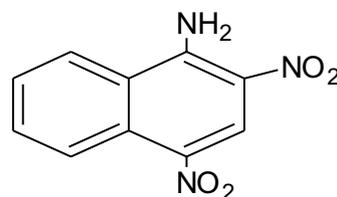
2-nitro-1-naphthol
(2-N1N) (III)



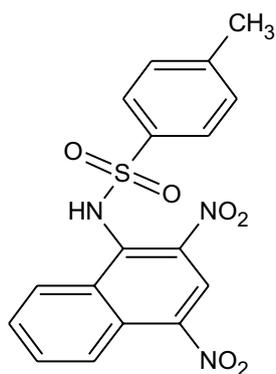
2,4-dinitro-1-naphthol
(2,4-DN1N) (IV)



4-nitro-1-naphthylamine
(4-N1NA) (V)



2,4-dinitro-1-naphthylamine
(2,4-DNINA) (VI)



N-(2,4-dinitro-1-naphthyl)-*p*-toluene
sulphonamide {N-(2,4-DN1NL) PTS}
(VII)

Fig. 3 Structures, names and abbreviations of the seven screened nitroaromatics

quinolones, respectively. This implies that this reagent supported charge transfer formation with the studied drugs.

The 1-naphthol set of nitroaromatics, 2, 4-DN1N and 2-N1N with two and one nitro groups as electron withdrawing substituents, respectively, also showed evidence of charge transfer formation as confirmed by the UV–VIS spectra of their complexes in Figs. 1b, c and 2b, c for cephalosporins and quinolones respectively, when compared with those of free reagents. Thus, these reagents formed charge transfer complexes with the studied drugs. The ability to form charge transfer complexes of these set of nitroaromatics could also be attributed to the presence of acidic hydrogen (-OH) in addition to the nitro groups. The oxygen atom of the hydroxyl group in 2, 4-DN1N and 2-N1N is more electronegative compared to the nitrogen atom of the amine group in 4-N1NA and 2, 4-DN1NA (Fig. 3). This being so, one or two nitro groups in either 2, 4-DN1N or 2-N1N was able to withdraw electrons from the ring rendering the hydrogen of the hydroxyl group relatively positive ($O^{\delta-}-H^{\delta+}$), i.e. acidic. 2, 4-DN1N and 2-N1N were then able to accept electrons from the studied drugs, forming charge transfer complexes with a bathochromic shift as shown by the spectra of adducts (Figs. 1, c, 2b, c). The charge transfer complex formation could also be seen as protonation of the primary and ring amino groups of cephalosporins and quinolones, respectively, by the acidic hydrogen (-OH) for example, of 2, 4-dinitro-1-naphthol [34].

The 1-naphthylamine set of nitroaromatics, 2, 4-DN1NA and 4-N1NA has in addition to the two and one nitro groups, respectively, an $-NH_2$ group in their molecules. Unlike the aforementioned set of nitroaromatics, the ultraviolet spectra of these complexes when compared with those of free reagents as shown in Figs. 1d, e and 2d, e, respectively, for cephalosporin and quinolones did not show bathochromic shifts, thus they could not form charge transfer complexes with the studied cephalosporins and quinolones. The effects of the electron withdrawing groups ($-NO_2$) in these molecules seemed not as pronounced because of less electronegativity of the nitrogen atom of the primary amine groups in 4-N1NA and 2, 4-DN1NA and thus, the hydrogen of the amine could not protonate the amino groups of the studied drugs for charge transfer complex formation.

The naphthalene set of nitroaromatics, 1, 4-DNNaph and 1-NNaph have only two and one nitro groups ($-NO_2$) as substituents, respectively. The UV–VIS spectra of their complexes compared with those of free reagents as shown in Figs. 1f, g and 2f, g, respectively, for cephalosporins and quinolones showed hypochromic effect and hypsochromic shifts, without bathochromic shift in either of the complexes. These observations imply that

these two reagents did not support charge transfer reaction with the studied cephalosporins and quinolones. The inability of these nitroaromatics to support charge transfer complex formation could be attributed to lack of effective acidic hydrogen in their molecules as explained for 2-N1N and 2, 4-DN1N.

Conclusions

The screening procedure carried out in this study has demonstrated the superiority of *N*-(2, 4-dinitro-1-naphthyl)-*p*-toluene sulphonamide and 2, 4-dinitro-1-naphthol over 2-nitro-1-naphthol amongst the three nitroaromatics that formed charge transfer complexes with the selected quinolones and cephalosporins. *N*-(2, 4-dinitro-1-naphthyl)-*p*-toluene sulphonamide and 2, 4-dinitro-1-naphthol could then be used as charge transfer reagents for identification and determination of the selected quinolones and cephalosporins. UV–VIS spectral analysis obtained from adducts and blank solutions of *N*-(2, 4-dinitro-1-naphthyl)-*p*-toluene sulphonamide and 2, 4-dinitro-1-naphthol illustrated its potential for quantitative estimation by absorption spectroscopy.

Abbreviations

μ m: Micrometer; °C: Degree Celsius; 1, 4-DNNaph: 1, 4-Dinitronaphthalene; 1-NNaph: 1-Nitronaphthalene; 2, 4-DN1N: 2, 4-Dinitro-1-naphthol; 2, 4-DN1NA: 2, 4-Dinitro-1-naphthylamine; 2-N1N: 2-Nitro-1-naphthol; 4-N1NA: 4-Nitro-1-naphthylamine; A: Acceptor molecule; D: Donor molecule; Abs: Absorbance; BP: British Pharmacopoeia; CFR: Ceftriaxone disodium salt; CFR-2, 4-DN1N: Ceftriaxone-2, 4-dinitro-1-naphthol adduct; CFR-*N*-(2, 4-DN1NL) PTS: Ceftriaxone-*N*-(2, 4-dinitro-1-naphthyl)-*p*-toluene sulphonamide adduct; CFT: Ceftazidime pentahydrate; CFT-2, 4-DN1N: Ceftazidime-2, 4-dinitro-1-naphthol adduct; CFT-*N*-(2, 4-DN1NL) PTS: Ceftazidime-*N*-(2, 4-dinitro-1-naphthyl)-*p*-toluene sulphonamide adduct; CFU: Cefuroxime sodium salt; CFU-2, 4-DN1N: Cefuroxime-2, 4-dinitro-1-naphthol adduct; CFU-*N*-(2, 4-DN1NL) PTS: Cefuroxime-*N*-(2, 4-dinitro-1-naphthyl)-*p*-toluene sulphonamide adduct; g: Gramme; hr: Hour; HPLC: High-performance liquid chromatography; IP: International pharmacopoeia; Kg: Kilogram; M.O.: Molecular orbital; mg: Milligramme; min: Minutes; mL: Millilitre; *N*-(2, 4-DN1NL) PTS: *N*-(2, 4-dinitro-1-naphthyl)-*p*-toluene sulphonamide; nm: Nanometer; NOR: Norfloxacin; NOR-2, 4-DN1N: Norfloxacin-2, 4-dinitro-1-naphthol adduct; NOR-*N*-(2, 4-DN1NL) PTS: Norfloxacin-*N*-(2, 4-dinitro-1-naphthyl)-*p*-toluene sulphonamide adduct; OFL: Ofloxacin; OFL-2, 4-DN1N: Ofloxacin-2, 4-dinitro-1-naphthol adduct; OFL-*N*-(2, 4-DN1NL) PTS: Ofloxacin-*N*-(2, 4-dinitro-1-naphthyl)-*p*-toluene sulphonamide adduct; p: Level of significance; RSD: Relative standard deviation; SD: Standard deviation; SPA: Sparfloxacin; SPA-2, 4-DN1N: Sparfloxacin-2, 4-dinitro-1-naphthol adduct; SPA-*N*-(2, 4-DN1NL) PTS: Sparfloxacin-*N*-(2, 4-dinitro-1-naphthyl)-*p*-toluene sulphonamide adduct; TLC: Thin-layer chromatography; USP: United state pharmacopoeia; UV: Ultraviolet–visible; ϵ : Molar absorptivity; NAC: Nitro aromatic compound; CNAC: Chloronitroaromatic compound; H_2SO_4 : Sulphuric acid; Conc.: Concentrated; ppt.: Precipitate; Eqn.: Equation; CTC: Charge transfer complex; E: Transition energy; f : Oscillator frequency; μ_{EN} : Transition dipole; R_N : Resonance energy; I_N : Ionisation potential; eV: Electron volt; W: Dissociation energy; K: Formation constant.

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Authors' contributions

OAA conceived the concept of the work and supervised it. OEU designed and carried out the laboratory experiments, obtained and analysed data and prepared a draft of the manuscript. DEE edited and prepared the final copy of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data and materials used to make inferences and conclusion of this work have been provided in the blinded copy. Any further data that may be requested shall be provided.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All the authors approved the manuscript for publication.

Competing interests

All authors declare no competing interests.

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