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4-Carboxyl-2,6-dinitrobenzene diazonium ion (CDNBD): a new diazonium for the detection of phenol ether homologues

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Abstract

Pharmaceutical phenol ethers are a heterogeneous group of compounds possessing ether linkage to an aromatic nucleus. Diazo coupling is rare with these compounds and no physicochemical method of analysis has been reported based on the reaction of these ethers with a diazonium ion. The high reactivity of 4-carboxyl-2,6-dinitrobenzene diazonium ion (CDNBD) is therefore investigated in this work. Comparative coupling reaction was done with two other diazonium ions (derived from *p*-nitroaniline and sulphanilic acid). Twenty-two phenol ethers were selected for evaluation of their reactivity with the three diazonium ions. Such ethers consist of those with naphthalenes, indole and bridged rings. Spot tests were used to establish coupling at room and elevated temperatures. Visual inspection and thin layer chromatographic (TLC) analysis of the reaction mixture provided evidence of coupling or otherwise. UV-VIS absorption spectra were used to characterize brightly coloured adducts, as a preliminary test for the estimation of these ethers by spectrophotometry. Of all the ethers screened, fourteen gave instant and distinct colour from CDNBD while seven of these gave colours of deeper intensity at elevated temperature. Only three of the ethers gave instant colour with diazotized p-nitroaniline while one compound gave instant colour with diazotized sulphanilic acid. UV absorption spectral analysis reveals the superiority of CDNBD for the detection and possible estimation of these ethers. CDNBD is shown to be a highly reactive arenediazonium ion with the possibility of finding usefulness for the determination of phenol ethers by ultraviolet/visible spectrophotometry and precolumn derivatization in high performance liquid chromatographic (HPLC) analysis.

Keywords: CDNBD, pharmaceutical phenol ethers, diazo coupling, *p*-nitroaniline, sulphanilic acid.

Introduction

Phenol ethers are also referred to as phenyl ethers or aryl ethers or phenolic ethers. Chemically, they consist of a phenyl or naphthyl ring with a methoxyl or an aryloxyl moiety attached. A number of naturally occurring phenol ethers are important constituents of volatile oils from various plants. They are used as food and pharmaceutical flavours, and in perfumery. They include anethole, eugenol and vanillin (Stenlake, 1979). A number of naturally occurring bases, including codeine, papaverine and tubocurarine, also may be regarded as complex examples of phenol ethers.

Heterogeneous group of drugs possessing a methoxy, ethoxy, alkoxyl or aryloxyl linkage to a phenyl or naphthyl ring were studied as potential couplers to three diazonium ions; the two well established diazonium ions (diazotized sulphanilic acid-DSPA and

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diazotized *p*-nitroaniline-DPNA) and the newly developed 4-carboxyl-2,6-dinitrobenzene diazonium ion (CDNBD). As at present, there is no general method for the determination of this heterogeneous class of drugs and particularly diazo coupling reaction. Besides the ether linkage, the ring is a second site of reaction in these ethers. The alkoxyl group, -OR, is *ortho*-, *para*-directing toward electrophilic aromatic substitution, and moderately activating (March, 1992). Alkoxy is a much stronger activator than alkyl, but much weaker than –OH (Saunders, 1949). The *ortho*- and *para*-positions are readily substituted and due to steric effects, *para*-substituted products are prevalent (Kohnstam and Williams, 1967).

Diazotized sulphanilic acid (Kozlov *et al.*, 1968) and diazotized p-nitroaniline (Smith and King, 1964) are among the earliest compounds used as reagents for activated skeletons either for identification as in the case of methoxamine with DPNA (BP 1993) or assay (Revanasiddappa and Manju, 2001; Prasad *et al.*, 2002). The weakly to moderately activating influence of ether linkage on aromatic skeletons when compared to an amine or a phenol narrows the range of diazonium ions that couple with such skeletons. 4-carboxyl-2,6-dinitrobenzene diazonium ion (CDNBD) was recently developed as a reagent for aromatic ring derivatization via the formation of a carbon-azo linkage to an aromatic nucleus and has been applied for UV-VIS analysis of drugs (Idowu, 1998; Idowu and Olaniyi, 2001; Idowu *et al.*, 2002; Idowu *et al.*, 2004). The exceptional reactivity of this diazonium is comparatively investigated in this work. The demonstration of the capability of this reagent to couple with this heterogeneous class will open a wide range of possible spectrophotometric and chromatographic analytical methods with a promise of ready availability.

Experimental

Chemical and reagents. Glacial acetic acid, ethyl acetate (99%), concentrated sulphuric acid, sodium nitrite crystals, orthophosphoric acid (all from BDH, Poole England), anhydrous magnesium sulphate, *p*-nitroaniline and sulphanilic acid (BDH). Drug samples screened are indomethacin, propranolol, nabumetone, nadolol, quinine, nafcillin, vanillin and reserpine (all chemical reference substances) as well as nimesulide, pindolol, naproxen, sodium cromoglicate, griseofulvin, astemizole, methocarbamol, trimethoprim, clomiphene, tamoxifen, bephenium hydroxynapthoate, penicillin V, sildenafil, dihydrocodeine tartrate extracted from dosage forms and recrystallised before use. Identity of the drugs were established by melting, TLC and IR spectroscopy in some cases. 4-amino-3,5-dintrobenzoic acid (synthesized in our laboratory).

Equipment. Analytical balance (Mettler H80, Leicester, U.K.), ultrasonic thermostated bath (Langford, Birmingham, UK), vortex mixer (Griffith and George ltd, UK), UV/VIS spectrophotometer (Unicam Aurora, Helios scan, v 1.1), ultraviolet lamp (254 and 365nm), silica gel GF_{254} precoated plates (G. Merck Germany).

Preparation of the diazonium ion reagent solutions.

(a) CDNBD: The reagent solution was routinely prepared from the assay kit (Idowu *et al.*, 2005) consisting of 0.125% w/v ADBA in Conc. H_2SO_4 (solution A), 5% w/v dried $NaNO_2$ in conc. H_2SO_4 (solution B), 85% orthophosphoric acid (solution C) and powdered urea (solute D).

(b) DPNA: 0.4g of p-nitro-aniline was dissolved in 60ml of 1M HCl with the aid of heat. The solution was cooled to 15^0C and a 10% w/v solution of sodium nitrite (prepared, immediately before use, by dissolving 10g of sodium nitrite in 100ml of distilled water) was

added until one drop of the mixture turn starch iodide paper blue. (BP 1998a)
(c) DSPA: 0.9g of sulphanilic acid was dissolved in a mixture of 30ml of 2M HCl and 70ml of water in a100ml volumetric flask. To 3ml of the solution, 3ml of a 5% solution of sodium nitrite was added and then cooled in ice for 5 minutes. 12ml of the sodium nitrite solution was added again and the mixture was cooled. The solution was then diluted to 100ml with water and the reagent kept in ice. (BP 1998b)

Preparation of stock solutions.
Each compound (5 mg) was dissolved in 1ml of glacial acetic acid.

Coupling reaction. The stock solution (0.2ml) of each compound was added to a test tube containing 0.5ml of the CDNBD solution. The test tube was shaken vigorously on a vortex mixer and the colour produced was observed within 5 minutes and recorded. This procedure was repeated with the other two diazonium ions (DPNA and DSPA).

Effect of temperature on coupling reaction.
Each reaction mixture was placed in a thermostated bath at 70^0C for 20 minutes. Any change in colour or appearance of turbidity was noted in addition to changes in intensity of colour in each tube at 5 and 20 minutes. In all cases, control reaction tubes were set up containing respective diazonium ions of CDNBD, DPNA and DSPA.

Processing of the reaction mixture.
The reaction mixtures were transferred into an ice-bath. 5ml of ice-cold water was added to the test tubes, shaken vigorously and extracted with 5ml ethylacetate with 50 inversions of the extraction tube at each extraction. The ethylacetate layer was transferred into a test tube, dried over anhydrous $MgSO_4$ and concentrated to a small volume. The concentrated extract was used for thin layer chromatographic analysis.

Thin layer chromatographic (TLC) analysis.
The TLC procedure was carried out on silica gel GF_{254} 0.2mm pre-coated plate using optimized mobile phase of ethylacetate : methanol (9:1) and ethylacetate : methanol (6:4). For each drug, four different solutions were spotted: the drug-reagent reaction mixture at room temperature; and at elevated temperature; solution of the drug in methanol; and the control reagent solution (CDNBD, DPNA or DSPA). Only reaction mixtures of drugs that produced colour at room and/or elevated temperature were considered for TLC analysis. The developed plates were air-dried and visualized in day-light and under UV lamp at 254nm. Spots identified under UV light were marked and the R_f values calculated.

UV-VIS absorption spectrum determination
A 1 mg/ml drug stock solution in glacial acetic acid was used for this study. The absorption spectrum measurement was done for drugs that produced coloured adducts with CDNBD and DPNA. A 0.1ml quantity of each drug stock solution was added into test tube containing 0.5 ml of CDNBD. The test tube was shaken vigorously on a vortex mixer for 10 seconds followed by incubation at 30°C for 20 minutes. The reaction was terminated by addition of 5ml of ice cold water to the reaction mixture in an ice-bath. The aqueous solution was extracted with 10ml of ethylacetate, and the extract was placed in a vial wrapped with aluminum foil. A blank reagent solution was prepared in a similar way, but the drug stock solution was replaced with glacial acetic acid.

Similar procedure was carried out for drugs that gave colours with DPNA. The absorption spectrum of the reaction mixture extract was determined by using ethyl acetate for acquiring the baseline. The absorption spectrum of the ethyl acetate extract of the adduct was overlaid on that of the blank reagent extract and absorption maximum (λ_{max}) was selected by inspection.

Results

Diazo coupling was carried out as described between the selected pharmaceutical phenol ethers (Table 1) and the three diazonium ions. All the drugs studied possessed some degree of activated skeletons. The results of the spot tests and chromatographic R_f values for the adducts of CDNBD and DPNA are presented in Tables 2 and 3 respectively while Table 3 shows the spot test results for DPSA. Good chromatographic resolutions were achieved using the less polar mobile phase (EtOAc: MeOH 9:1).

The absorption spectra of the adducts formed between CDNBD and propranolol, indomethacin, nadolol, naproxen, nabumetone and reserpine were recorded and presented in Figures 1-6 respectively, while those between DPNA and nafcillin, vanillin, naproxen and nabumetone are presented in Figures 7-10.

Discussion

One major feature of this screening procedure was the fast speed with which CDNBD reacted with the phenol ethers. The reaction was not as fast with DPNA and only one drug (vanillin) gave coloured adduct. Phenol ethers are weakly activated molecules and diazo coupling reactions fail between prototype arenediazonium ions and phenol ethers. An exceptionally reactive diazonium ion, (2,4-dinitrobenzene diazonium ion) is reported to couple with some phenol ethers (anisole and phenetole), although it was not applied as a reagent. Diazonium ions like *p*-nitrodiazobenzene do not form azo dyes with monohydric phenol ethers like anisole but with resorcinol ethers and better still with phloroglucinol ethers. (Meyer *et al.*, 1914; Saunders, 1949).

The instant colour obtained with majority of the pharmaceutical phenol ethers screened lends credence to the exceptional reactivity of CDNBD, which is also a dinitrobenzene diazonium analog. The presence of an additional electron withdrawing carboxylic acid moiety *para* to the diazo group further enhances its reactivity.

Of the twenty-two pharmaceutical phenol ethers screened, fourteen gave instant and distinct colours different from the colour produced with blank reagent medium (control). However, only six of these gave colours of deeper intensity at elevated temperature.

The naphthalene derivatives (propranolol, naproxen, nabumetone) gave instant colours which were quite distinct from the blank reagent. It was discovered for propranolol and naproxen that the colour change progressed through various shades until a reddish-pink colour was observed. This might be due to sequential formation of intermediates in the process of forming the most stable adduct. The two other β-adrenergic receptor blockers screened, pindolol and nadolol, gave different colours. While pindolol reacted to give a purplish-brown colour using tablet extract (Visken® in methanol), only a reddish-brown colour was obtained for pure pindolol crystals isolated by gradient elution form a flash chromatographic run. Both samples of pindolol however produced a light-purple colour with the acidic blank medium. Thus, investigation with pindolol was not pursued any further as the medium will be contributing a great deal to the absorptivity of the adduct. The reaction may be due to the indole nucleus. The reaction with the blank medium was however not observed with reserpine, another indole, screened. The reactivity of reserpine with the reagent might receive additional contribution from the ring bearing the trimethoxyl moieties. The reddish-brown colour obtained was maintained at elevated temperature and changed to deep orange on adding water and in ethylacetate.

Nadolol, being a tetrahydro-naphthalene diol did not give an observable colour

immediately. It took about 60 seconds before the pink colour appeared. It is most likely that dehydration of the diol moiety took place during the time lapse. Incubation at elevated temperature further enhanced the colour observed. The dehydration taking place *in situ* will further reduce any error that may be incurred in trying to carry out a prior step before coupling. As the reaction medium contains 1:2 ratio of concentrated H_2SO_4 to orthophosphoric acid, the dehydration step should proceed faster, since the latter is known to effect the step better than H_2SO_4 that may produce some charring. Apart from the naphthalenes (naproxen and nabumetone), molecules that do not have substituents *para* to the ether linkage freely produced instant colours. Other molecules with no substituents *ortho* to the ether linkage did not readily react to give brilliant colours. Thus, molecules like tramadol, mepyramine, dihydrocodeine, clomifene and tamoxifen gave colours that were barely deeper than the control. This indicates that coupling at *ortho* position may be significantly hindered within these molecules.

Substitution of the reagent on griseofulvin molecules seem to proceed with only a little change in the λ_{max} as the deep golden yellow colour gave a λ_{max} of 325nm. There may not be any chromophoric elongation or a difficulty of substitution in between the methoxyl groups. The possibility of two molecules of the reagent attacking the ring simultaneously was not noticed especially for drugs like methocarbamol and the flavouring agent, vanillin. When such a step is favoured, an elongation of wavelength of absorption might be observed. On UV-VIS spectrophotometric scanning, the adduct of methocarbamol gave a new λ_{max} at 336nm as against the 270nm of the drug. Substitution by a second mole of the reagent may actually be hindered as the ring is already deactivated on substitution by the first molecule. Vanillin does not appear stable within the medium.

Nimesulide and bephenium, which are phenoxyl ethers, did not produce any appreciable colour with the reagent. While bephenium gave light yellow colour, the orange colour observed for nimesulide was only obtained at elevated temperatures. This points to a weak-activating potential of the phenoxyl group as compared to methoxy, ethoxy and aryloxyl propanolamine. A hyperchromic shift was observed for the adduct of nimesulide (296nm – 0.486) compared to pure drug (300nm – 0.208) both at 10μg/ml concentration.

Based on the screening of these representative phenol ethers, certain generalizations may be articulated with regards to their reactivity with CDNBD.

(i) α-Naphthol ethers such as propranolol and nadolol produced instant colours. It is expected that substitution will be at position 4, on the ring, *para* to the ether linkage.

(ii) Naphthalene ethers like naproxen and nabumetone produced instant colours, giving rather exceptional result of *ortho* or *meta* coupling to the methoxy groups.

(iii) Indole ethers screened gave different results and it appears that the presence of methoxyl groups on the benzene residue (reserpine and indomethacin) makes them susceptible to electrophilic substitution than in pindolol with an aryloxy propanolamine side chain.

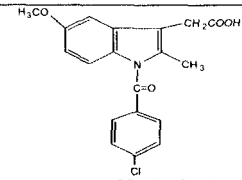
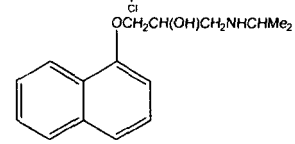
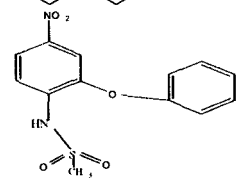
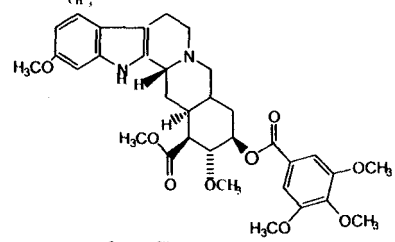
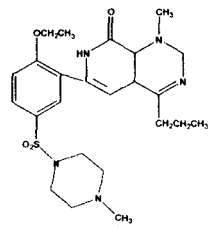
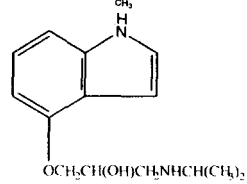
(iv) Ethers in which only the *ortho* position is free react with difficulty and merely gave hyperchromic shift (e. g. clomifene and tamoxifen).

(v) Phenoxyl ethers also react with extreme difficulty. This point to a weak activating property of the oxygen atom (e. g. nimesulide and bephenium).

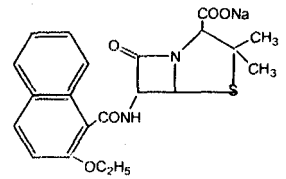
CDNBD however seem to be a good reagent for the qualitative estimation of phenol ethers. The single nitro group on DPNA makes it weakly deactivated and less reactive than CDNBD. The presence of the $-SO_3$ on DSPA makes it the most weakly deactivated and

hence least reactive of the three. The superiority of CDNBD was further corroborated using the TLC and absorption spectra measurements. Single and well distinct spots are observed for the CDNBD adducts (Table 2) when compared with DPNA (Table 3). From figures 1-10, clear evidence is presented showing CDNBD a better derivatizing reagent than DPNA. This is because of distinguishable chromophoric elongation observed for CDNBD adducts. The suitability of CDNBD has been demonstrated in the analysis of propranolol tablets (Idowu et al, 2004)) and four other phenol ethers (Adegoke, 2005). A promising advantage of the investigative reagent (CDNBD) is the possibility of its application to high performance liquid chromatographic (HPLC) analysis by precolumn derivatization for virtually all of these ethers.

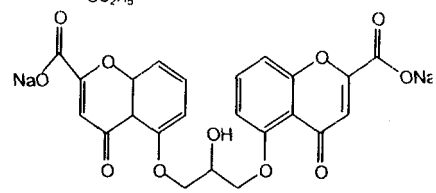
Table 1: Chemical structures and pharmacological action of drugs studied

S/N	Name and pharmacological action	Chemical structure
1.	Indomethacin (anti-inflammatory, antipyretic, analgesic)	
2.	Propranolol (antihypertensive)	
3.	Nimesulide (anti-inflammatory, antipyretic, analgesic)	
4.	Reserpine (antihypertensive)	
5.	Sildenafil citrate (Anti-fertility)	
6.	Pindolol (antihypertensive)	

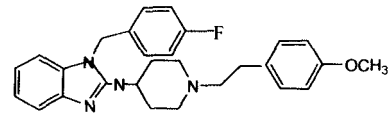
7. Nafcillin sodium (antibacterial)



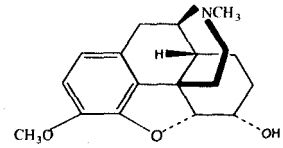
8. Sodium cromoglicate (antiasthmatic, antiallergic)



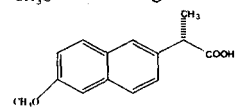
9. Astemizole (antihistamine)



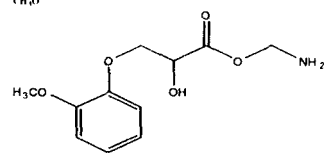
10. Dihydrocodeine (narcotic analgesic)



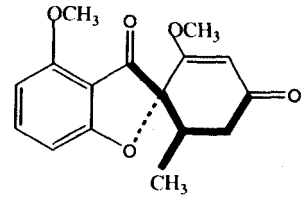
11. Naproxen (nonsteroidal antiflammatory drug)



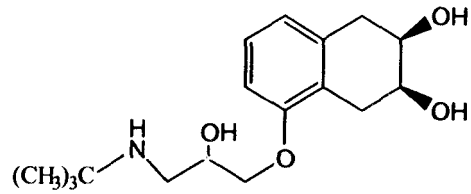
12. Methocarbamol (spasmolytic-skeletal muscle relaxant)



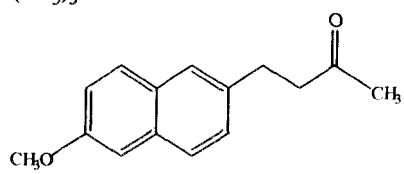
13. Griseofulvin (antifungal)



14. Nadolol (antihypertensive)



15. Nabumetone (nonsteroidal antiflammatory drug)



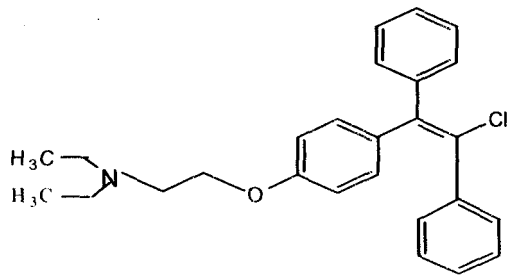
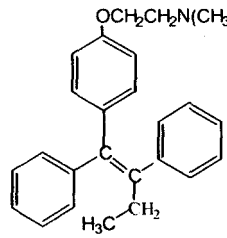
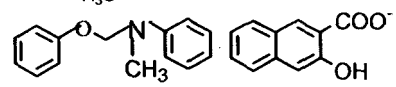
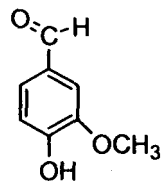
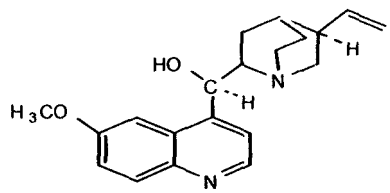
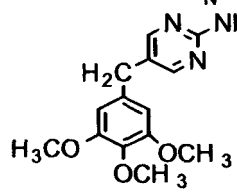
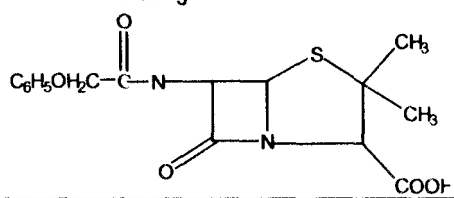
16.	Clomiphene (anti-oestrogen)	
17.	Tamoxifen (Anti-oestrogen)	
18.	Bephenium hydroxynaphthoate (antihelminthic)	
19.	Vanillin (flavouring agent)	
20.	Quinine (antimalarial, antiarrhythmic)	
21.	Trimethoprim (Antibacterial)	
22.	Penicillin V (antibacterial)	

Table 2: Spot test results and Chromatographic R_f values for CDNBD-azo adduct formed with drugs studied*

Drug	Colour at room temperature	Colour at 70°C (20 minutes)	R_f Values** (EtOAc:MeOH 9:1)
Indomethacin	Purple (instantly)	Dark purple	0.22 (0.81)
Propranolol	Instant blue to purple to reddish-pink	Turbidity and light pink	0.44 (0.09)
Nimesulide	No colour	Orange colour	0.63 (0.42)
Reserpine	Reddish brown colour	Colour maintained	0.31 (0.57)
Sildenafil	Light yellow	Colour maintained	-
Pindolol	Purplish-brown colour	Colour maintained	0.80 (0.1)
Nafcillin sodium	Instant deep golden yellow	Colour maintained	0.60 (0.58)
Sodium cromoglicate	Instant deep yellow	Colour maintained	0.68 (0.50)
Astemizole	Instant pink then to orange	Colour faded	0.61 (0.47)
Dihydrocodeine tartrate	Deep orange colour	Maintained	-
Naproxen	Instant blue to purple colour	Colour slightly discharged	0.68 (0.80)
Methocarbamol	Orange-brown colour	Deeper colour	0.67 (0.57)
Griseofulvin	Light golden yellow colour	Colour became deeper	0.78 (0.80)
Nadolol	Pink colour after 1 minute	Pinkish-orange colour	0.78 (0.13)
Nabumetone	Instant purple colour	Colour maintained	0.80 (0.88)
Clomiphene	Light pink	Colour deepened	0.59 (046)
Tamoxifen	Yellow colour	Deeper	0.68 (0.48)
Bephenium hydroxynaphthoate	Light yellow	Deeper	0.55 (0.34)
Vanillin	Light yellow	Colour maintained	0.68 (0.73)
Quinine	Light yellow	Light yellow	- (0.30)
Trimethoprim	Light yellow	Golden yellow	0.70 (0.10)
Penicillin V	Light yellow	Light yellow	-

** Blank reagent solution without drug is light yellow; ** R_f of drug substance in parenthesis (R_f of reagent= 0.55)*

Table 3: Spot test results and Chromatographic R_f values for DPNA-azo adduct formed with drugs studied*

Drug	Colour at room temperature	Colour at 70°C (20 minutes)	R_f Values** (EtOAc:MeOH 9:1)
Indomethacin	Cream (cloudy instantly)	Yellowish orange	0.77(0.76)
Propranolol	Yellow	Golden yellow	0.71(0.57)
Nimesulide	Cloudy	Light yellow	0.63(0.50)
Reserpine	Light yellow	Light yellow	-
Sildenafil	Greenish yellow	Light green	-
Pindolol	Yellow	Yellow	0.80 (0.10)
Nafcillin sodium	Yellow(cloudy)	Golden yellow	0.60 (058)
Sodium cromoglicate	Light yellow	Light yellow with precipitate	0.68 (0.66)
Astemizole	Cloudy	Cloudy	0.61(047)
Dihydrocodeine Citrate	Light yellow	Light yellow	-
Naproxen	Orange	Orange (turbid)	0.68 (0.80)
Methocarbamol	Yellow	Very light yellow	0.64 (0.57)
Griseofulvin	Light yellow	Light yellow	0.78 (0.80)
Nadolol	Light yellow	Light yellow	0.60 (0.13)
Nabumetone	Light pink	Blood-red (turbid)	0.74 (0.70)
Clomiphene	Light yellow	Very light yellow	-
Tamoxifen	Light yellow	Yellow	-
Bephenium hydroxynaphthoate	Greenish yellow	Yellow	0.30 (0.34)
Vanillin	Golden yellow	Deep yellow	0.68 (0.73)
Quinine	Light yellow	Light yellow	-
Trimethoprim	Light yellow	Golden yellow	0.70 (0.10)
Penicillin V	Light yellow	Yellow	-

** Blank reagent solution without coupling component is light yellow; ** R_f value of drug substance in parenthesis*

Table 4: Spot test results for DSPA-azo adduct formed with drugs studied*

Drug	Colour at room temperature	Colour at 70°C (20 minutes)
Indomethacin	Cloudy (with particles)	Yellow (cloudy)
Propranolol	Colourless	Greenish yellow
Nimesulide	Cloudy (with particles)	Light yellow
Reserpine	Colourless	Colourless
Sildenafil	Colourless	Colourless
Pindolol	Light yellow	Golden yellow
Nafcillin sodium	Colourless	Light yellow
Sodium cromoglicate	Colourless	Light yellow
Astemizole	Cloudy	Cloudy
Dihydrocodeine citrate	Colourless	Colourless
Naproxen	Colourless	Light greenish yellow
Methocarbamol	Colourless	Very light yellow
Griseofulvin	Colourless	Colourless
Nadolol	Colourless	Very light yellow
Nabumetone	Forms precipitate	Light greenish yellow
Clomiphene	Colourless	Light yellow
Tamoxifen	Colourless	Light yellow
Bephenium hydroxynaphthoate	Golden yellow	Greenish yellow
Vanillin	Green	Golden yellow
Quinine	Colourless	Light yellow
Trimethoprim	Colourless	Very light yellow
Penicillin V	Colourless	Light yellow

** The blank reagent without any drug is colourless*

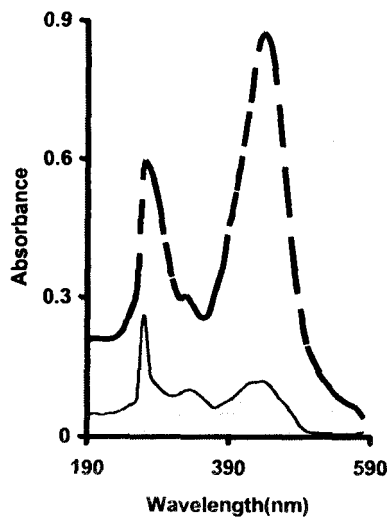


Figure 1: Absorption spectrum of propranolol-CDNBD azo adduct (-----) overlaid on that of the reagent (____)

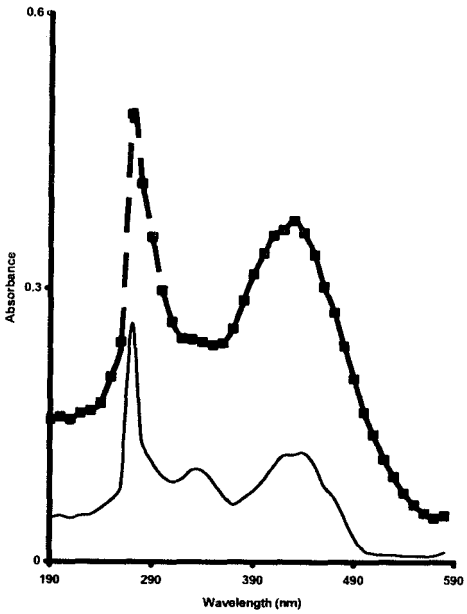


Figure 2: Absorption spectrum of indomethacin-CDNBD azo adduct (------) overlaid on that of the reagent (___)

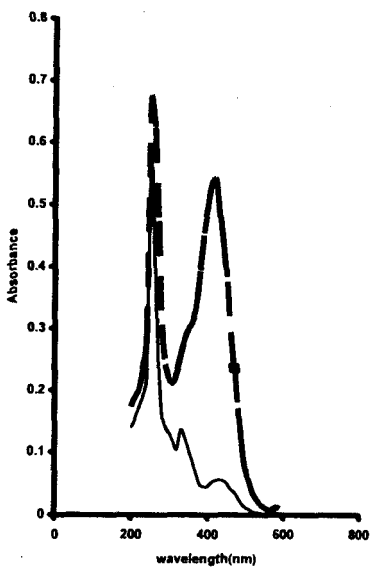


Figure 3 : Absorption spectrum of nadolol-CDNBD azo adduct (----) overlaid on that of the blank reagent (____)

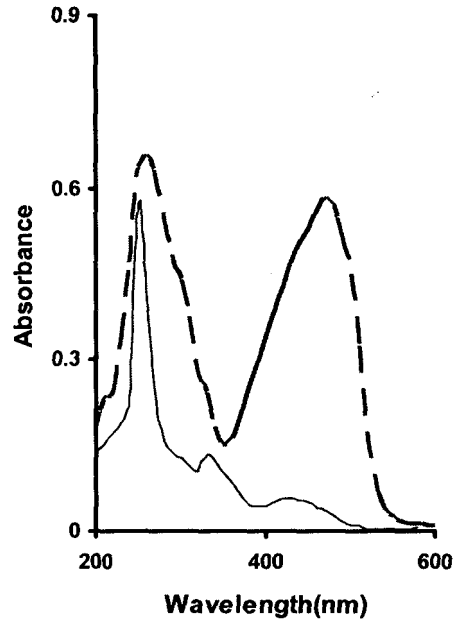


Figure 4: Absorption spectrum of naproxen-CDNBD azo adduct (------) overlaid on that of the blank reagent (____)

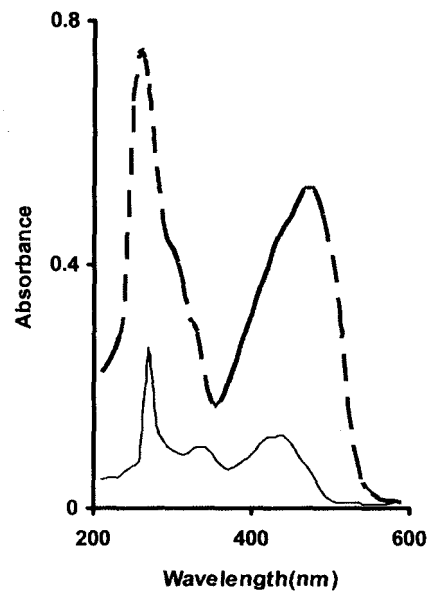


Figure 5 : Absorption spectrum of nabumetone-CDNBD azo adduct (----) overlaid on that of the blank reagent (____)

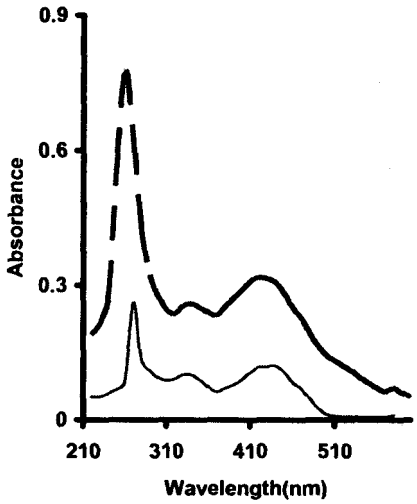


Figure 6: Absorption spectrum of reserpine-CDNBD azo adduct (-------) overlaid on that of blank reagent (____)

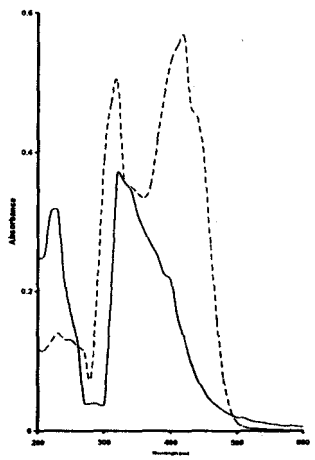


Figure 7: Absorption spectrum of nafcillin-DPNA adduct (-----) overlaid on that of DPNA (_____)

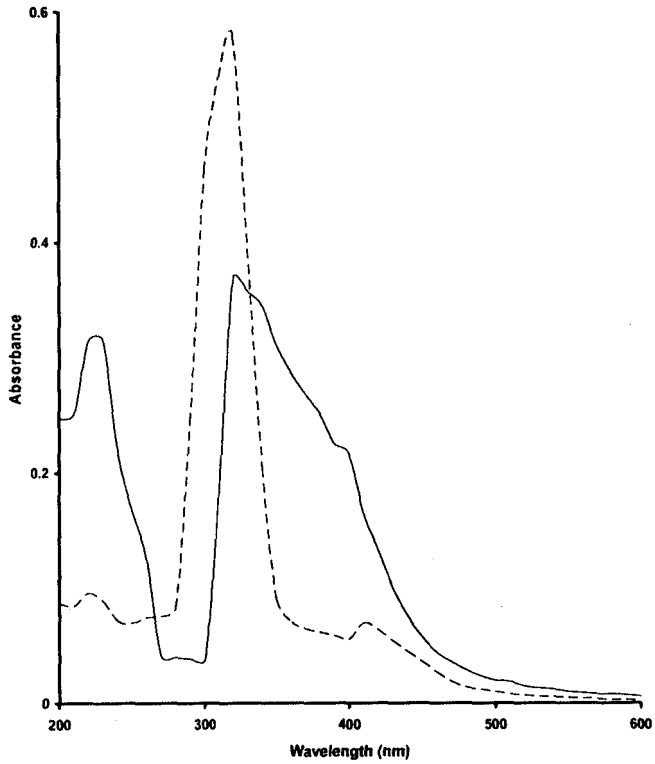


Figure 8: Absorption spectrum of vanillin-DPNA adduct (----) overlaid on that of DPNA (____)

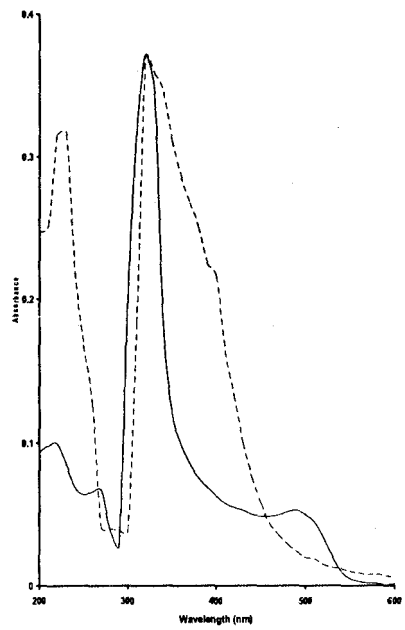


Figure 9: Absorption spectrum of naproxen-DPNA adduct (----) overlaid on that of DPNA (____)

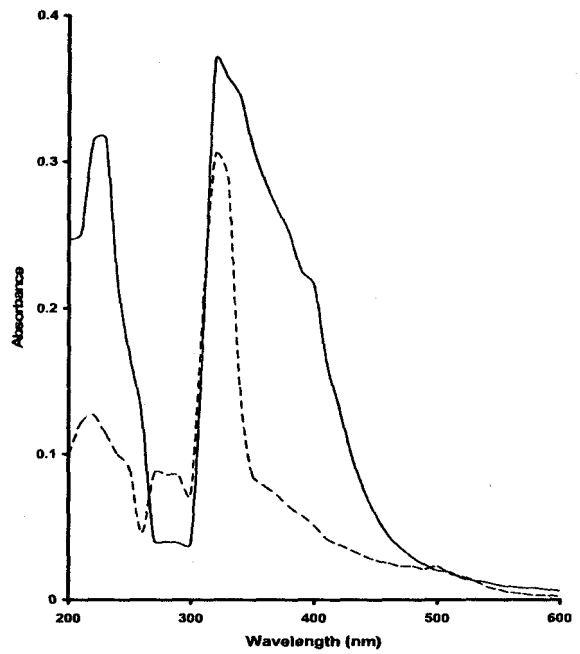


Figure 10: Absorption spectrum of nabumetone-DPNA adduct (----) overlaid on that of DPNA (____)

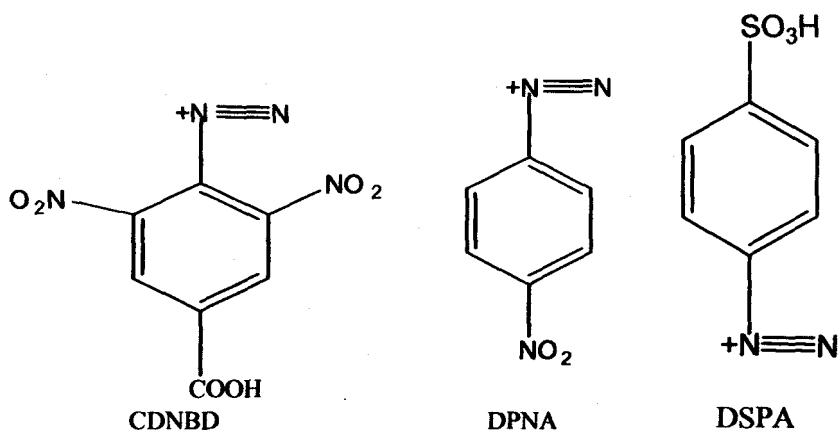


Figure 11: Structures of three diazonium ions studied

Conclusion

The screening procedure carried out in this work has demonstrated the superiority of 4-carboxyl-2,6-dintrobenzene diazonium ion (CDNBD) as a novel derivatizing reagent for the identification of pharmaceutical phenol ethers. Spectral analysis by UV-VIS spectroscopy illustrates its potential for quantitative estimation by absorption spectroscopy and precolumn derivatization on HPLC/ UV determination.

References

Adegoke A. O. (2005): Novel colorimetric assays of some selected pharmaceutical phenol ethers using 4-carboxyl-2,6-dinitrobenzene diazonium ion (CDNBD). Ph.D. Thesis. University of Ibadan.

BP 1993 Vol. II Her Majesty's Stationery Office, London. p.1006.

BP 1998a Vol. II Her Majesty's Stationery Office, London; p. 81

BP 1998b Vol. II Her Majesty's Stationery Office, London; p. 83

Idowu S.O. (1998): Development and Evaluation of 4-amino-3,5-dinitrobenzioc acid as a novel derivatizing reagent. Ph.D. Thesis, University of Ibadan

Idowu S.O. and Olaniyi A.A. (2001): Evaluation of diazotized 4-amino-3,5-dinitrobenzioc acid (ADBA) as a new derivatizing reagent, *Afr. J. Med. Sci.* 30 (3), 217-220.

Idowu S.O., Tambo S.C., Adegoke A.O. and Olaniyi A.A. (2002): Novel Colorimetric assay of mefenamic acid using 4-amino-3,5-dinitrobenzioc acid (ADBA), *Trop. J. Pharm. Res.* 1 (1), 15-22.

Idowu, S.O., Adegoke, A.O. and Olaniyi, A.A. (2004): Colorimetric assay of propranolol tablets by derivatization: Novel application of diazotized 4-amino-3,5-dinitrobenzoic acid (ADBA), *J. AOAC. Int.* 87(3), 573 – 578.

Idowu, S.O.; Kolawole, A. O.; Adegoke, A.O.; Kolade, Y.T.; Fasanmade, A.A. and Olaniyi, A.A. (2005); Kinetics of thermal decomposition of 4-carboxyl-2,6-dinitrobenzenediazonium ion (CDNBD); *J. AOAC Int.* 88 (4), 1108-1113

Kohnstam G., Williams D.L.H. (1967): Directing and activating effects of alkoxyl and aryloxy groups in aromatic and aliphatic reactions. In: The chemistry of the ether linkage, Patai S. (Ed) Interscience. pp. 82, 132-133.

Kozlov V.V., Silaeva T.D. and Eremin S.K. (1968): Diazo compounds XXXVII. Diazotization of aromatic amines in metaphosphoric acid. *Zh. Org. Khim.*; 4 (12): 2145-2149. {Chemical abstract: 67784X 1969, 70(4)}.

March J. (1992): Advanced Organic Chemistry: Reactions, Mechanisms and Structure. 4th Ed. John Wiley & Sons, N.Y. pp. 723-725

Meyer K.H., Irschick, A. & Schlosser, H. (1914): *Berichte der deutschen chemischen gesselscheft*; 47, 1741-1755.

Prasad A.V.S.S, Lakshmi C.S.R., Sastry C.S.P. and Uppuleti V.P. (2002): Determination of minocycline by oxidative coupling and diazocoupling reactions in pharmaceutical formulations, *J. Pharm. Biomed. Anal.* 30, 491-498.

Revanasiddappa H.D. and Manju B. (2001): Spectrophotometric methods for the determination of ritodrine HCl and its application to pharmaceutical preparations, *Il Farmaco* 56, 615-619.

Saunders K.H. (1949): The aromatic Diazo-compounds and their technical applications. 2nd ed. Edward Arnold & Co., London. P. 198

Smith G.A.L. and King D.A. (1964): Determination of steam-volatile phenols present in cigarette-smoke condensate; *Analyst* 89, 305-311.

Stenlake J.B. (1979) Foundations of molecular pharmacology. Vol. I Medicinal and Pharmaceutical Chemistry. The Athlone Press, London. Pp. 179-182