# Dediazoniation of *p*-hydroxy and *p*-nitrobenzenediazonium ions in an aqueous medium: Interference by the chelating agent diethylenetriaminepentaacetic acid

**B. Quintero, M.C. Cabeza, M.I. Martínez, P. Gutiérrez, and P.J. Martínez**

**Abstract:** We have made a comparative study of the dediazoniation of *p*-hydroxy and *p*-nitrobenzenediazonium ions. The electron-withdrawing and donating properties of the -$NO_2$ and -OH groups strongly determine the reactivity of both compounds, thus exerting different influences upon the dediazoniation reaction. We describe here how the decomposition of *p*-hydroxy and *p*-nitrobenzenediazonium ions in a neutral aqueous medium follows a different pattern in the presence of the metal-chelator diethylenetriaminepentaacetic acid (DTPA). The decomposition rate of *p*-hydroxybenzenediazonium decreases whilst the decomposition of the *p*-nitrobenzenediazonium ion is enhanced. The experimental data are discussed with reference to a common scheme of interference for both benzenediazonium ions in the light of the radical-scavenging capacity of DTPA.

*Key words:* *p*-hydroxybenzenediazonium ion, *p*-nitrobenzenediazonium ion, di-ethylenetriaminepentaacetic acid, dediazoniation, radical scavenging, artifacts.

**Résumé :** On a réalisé une étude comparative des réactions de dédiazotation des ions *p*-hydroxy et *p*-nitrobenzènediazonium. Les propriétés électroaffinitaires et électrorépulsives des groupes -$NO_2$ et -OH influencent fortement la réactivité de ces deux composés et exercent donc des influences différentes sur leur réaction de dédiazotation. La décomposition des ions *p*-hydroxy- et *p*-nitrobenzènediazonium dans un milieu aqueux neutre, en présence de l'acide diéthylènetriaminepentaacétique (DPTA) qui agit comme chélatant de métaux se produit selon des voies différentes. La vitesse de décomposition de l'ion *p*-nitrobenzènediazonium diminue alors que celle de l'ion *p*-hydroxynitrobenzènediazonium augmente. On discute des données expérimentales en fonction d'un schéma commun d'interférence pour les deux ions benzènediazonium et en tenant compte de la capacité du DTPA à piéger les radicaux.

*Mots clés :* ion *p*-hydroxybenzènediazonium, ion *p*-nitrobenzènediazonium, acide diéthylènetriaminepentaacétique, dédiazotation, piège de radicaux, artefacts.

[Traduit par la Rédaction]

## Introduction

Arenediazonium ions are widely used in chemical synthesis. Apart from diazo-coupling reactions (1–3), these ions can undergo either thermal or photochemical heterolytic dediazoniation, yielding the aryl cation (1, 4, 5). Arenediazonium ions can also be dediazoniated in a homolytic process via one-electron reduction, thus generating aryl radicals. Arenediazonium ions and their precursors, arylhydrazides and arylhydrazines, are believed to be genotoxic (6, 7). Although most arenediazonium ions are recognised as being oxidants, such structural characteristics as the electron-donating and withdrawing properties of the substituents in the aromatic ring, together with interference from solvent interactions, other reducing and (or) oxidizing compounds, light, or the conditions of the reaction medium may substantially modify their reactivity, giving rise to different patterns of decomposition and resulting in different biological effects. In this context heterolytic dediazoniation has been reported to occur with methylbenzenediazonium and *p*-nitrobenzenediazonium (pNO) ions in an aqueous medium (8, 9), whilst other authors have interpreted their results as showing evidence of heterolytic and homolytic processes during the thermal and photochemical dediazoniation of several arenediazonium ions in trifluoroethanol and ethanol (10, 11). Furthermore, in certain cases the reducing capacity of water has been sufficient to reduce arenediazonium to the aryl radical (12). It is obvious therefore that reaction conditions need to be chosen carefully when trying to establish the mechanisms involved in the decomposition of such versatile compounds as arenediazonium ions. We have studied the dediazoniation of the *p*-hydroxybenzenediazonium ion (PDQ) in a neutral aqueous medium (13) and have observed that in the presence of DTPA the rate of dediazoniation decreases together with the intensity of the signal produced by the aryl radical adduct as measured in EPR, using the spin

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trap DMPO (5,5-dimethyl-1-pyrroline-*N*-oxide). The use of DTPA as metal chelator has been recommended in the literature to avoid interference from the redox activity of any contaminating metal ions present in solution (14–17). We report here on a comparative study carried out with pNO and PDQ, the dediazoniation rates of which are influenced differently by the chelating agent DTPA.

## Experimental

Chemicals of the highest available purity (Merck and Aldrich) were used. Chelex 100 resin (50–100 dry mesh, sodium form), nitrobenzene, *p*-nitrophenol, and pNO tetrafluoroborate were bought from Sigma and used as received. PDQ tetrafluoroborate was synthesized following the procedure described by Danêk et al. (18) with slight modifications. Sodium tetrafluoroborate (2.24 g) in distilled water (6 mL) was treated with perchloric acid (70%, 1.7 mL) and used to dissolve *p*-aminophenol (1.09 g) (Solution 1). Another solution was made by dissolving sodium nitrite (0.71 g) in distilled water (2.5 mL) (Solution 2). Solution 2 was added gradually to Solution 1, while stirring continuously and keeping the reaction in darkness within a temperature range of 0–5°C. The resulting mixture was kept at –10°C for 24 h. A solid was separated by filtration and washed first with cold ethanol and then with diethyl ether. Crystallization was made by precipitation from the solution obtained, dissolving the solid in ethanol at 70°C. A second crystallization was carried out by adding diethyl ether to an acetone solution of the solid. Re-crystallized PDQ tetrafluoroborate is a yellowish crystalline solid that melts between 135 and 140°C accompanied by a noticeable change in colour and the production of a gas. Elemental analysis revealed C 42.8%, H 2.76%, and N 17.05%, which agrees very well with the formation of the tetrafluoroborate of the PDQ dimer. IR: 2189 $cm^{-1}$ (N≡N stretching) and 1591 $cm^{-1}$ (aromatic group). Both benzenediazonium salts were stored below –18°C in darkness.

A Hucoa Erlöss Cintra 10 spectrophotometer was used for spectrophotometric analysis. HPLC was done with a Merck L-6220 biocompatible pump and a Merck L-4500 diode array detector (Merck-Hitachi). Aqueous media were filtered through Millipore HA filters with a pore size of about 0.45 μm. The column was a Spherisorb ODS-2 (4.6 mm × 200 mm) with a particle size of 5 μm. Mobile phase acetonitrile–methanol–acetic acid (1%) (30:30:40) with a flow of 0.7 $mL \cdot min^{-1}$ was routinely used. Samples were dissolved in phosphate buffer (0.1 M, pH 7.2) previously treated with Chelex 100 resin by the column method. A Radiometer pH M64 potentiometer with a GK2401C mixed electrode was used whenever called for. The calibrations were carried out with Crison buffer references (pH 4 and pH 7). pH values were checked throughout the kinetic measurements, and no significant changes were observed. An oxygraph equipped with a Clark-type electrode was used to measure oxygen consumption. Twice-distilled water was obtained by the Milli Q system and used in all experiments. Oxygen for deaerated samples was purged by bubbling with argon for at least 10 min.

We checked for any effects on PDQ and pNO decomposition that might be caused by either environmental laboratory light or apparatus light sources. The results were taken into account when designing the methods for spectrophotometric measurement. In the case of PDQ, measurements were routinely made with aliquots taken from a stock solution kept in darkness. Neither environmental nor instrumental light interference was observed in the case of pNO.

Kinetic analyses were made by incubating 0.4 mM PDQ solutions kept in darkness at 37°C either in the presence or absence of DTPA. Aliquots were taken from these solutions to make PDQ 0.01 mM solutions, which were then used for spectrophotometric measurements. Kinetic measurements were made with pNO in the presence or absence of DTPA either by incubating 1.33 mM pNO solutions and then taking aliquots to make $3.26 \times 10^{-5}$ M pNO solutions or by placing the sample (0.11 mM) directly into the spectrophotometric cell.

## Results and discussion

### Dediazoniation of PDQ and pNO in the absence of DTPA

The absorption spectrum of PDQ in a phosphate-buffered aqueous medium (pH 7.2) presented a band with its maximum at 350 nm (ε : 41 990 $L \cdot mol^{-1} \cdot cm^{-1}$) and a less intense band at 250 nm (ε : 3010 $L \cdot mol^{-1} \cdot cm^{-1}$), whereas the absorption spectrum of pNO obtained in an identical medium showed a band with its maximum at 259 nm (ε : 15 590 $L \cdot mol^{-1} \cdot cm^{-1}$) and a minor absorption at 314 nm (ε : 2234 $L \cdot mol^{1} \cdot cm^{-1}$).

We have reported previously (13) that, under experimental conditions controlled to prevent photochemical and (or) heterolytic side reactions, the dediazoniation of PDQ in a neutral aqueous medium (37°C in phosphate buffer, pH 7.2) occurs via three pathways (Scheme 1): Pathway **1** represents dediazoniation induced by a hydroxyl ion, a slow process at neutral pH and even slower with deaerated samples. In pathway **2** the formation of a semiquinone radical via the reaction of an aryl radical with oxygen is considered to justify the increase in the dediazoniation rate in the presence of oxygen. Finally, in pathway **3**, hydroquinone, produced by semiquinone dismutation, may act as an additional reducing agent. PDQ dediazoniation was characterized by a gradual decrease in absorbance at 350 nm. pNO dediazoniation, on the other hand, using a sample concentration of 1.33 mM, led to a decrease at 259 nm followed by the simultaneous appearance of an absorption band at 350 nm, which increased with time. No band was recorded beyond 370 nm (Fig. 1). As the concentration of pNO fell (0.11 mM), the picture changed, with a new band appearing at about 390 nm, as well as the absorption at 350 nm (Fig. 2). A chromatographic analysis of these samples containing a low concentration of pNO was made using acetonitrile–methanol–acetic acid 0.2 M (30:30:40) as the mobile phase. The chromatograms showed three main peaks with retention times of 3.05, 4.01, and 7.26 min, respectively, and a minor peak at 10.41 min, which, by comparing the associated UV spectra with those of the authentic products, were assigned to pNO, PDQ, *p*-nitrophenol (pNP), and nitrobenzene (NB), respectively. The unexpected presence of NB led us to repeat the HPLC analysis using fresh samples of pNO in $1 \times 10^{-4}$ M HCl at 25°C, which should be very stable according to the

**Scheme 1.**

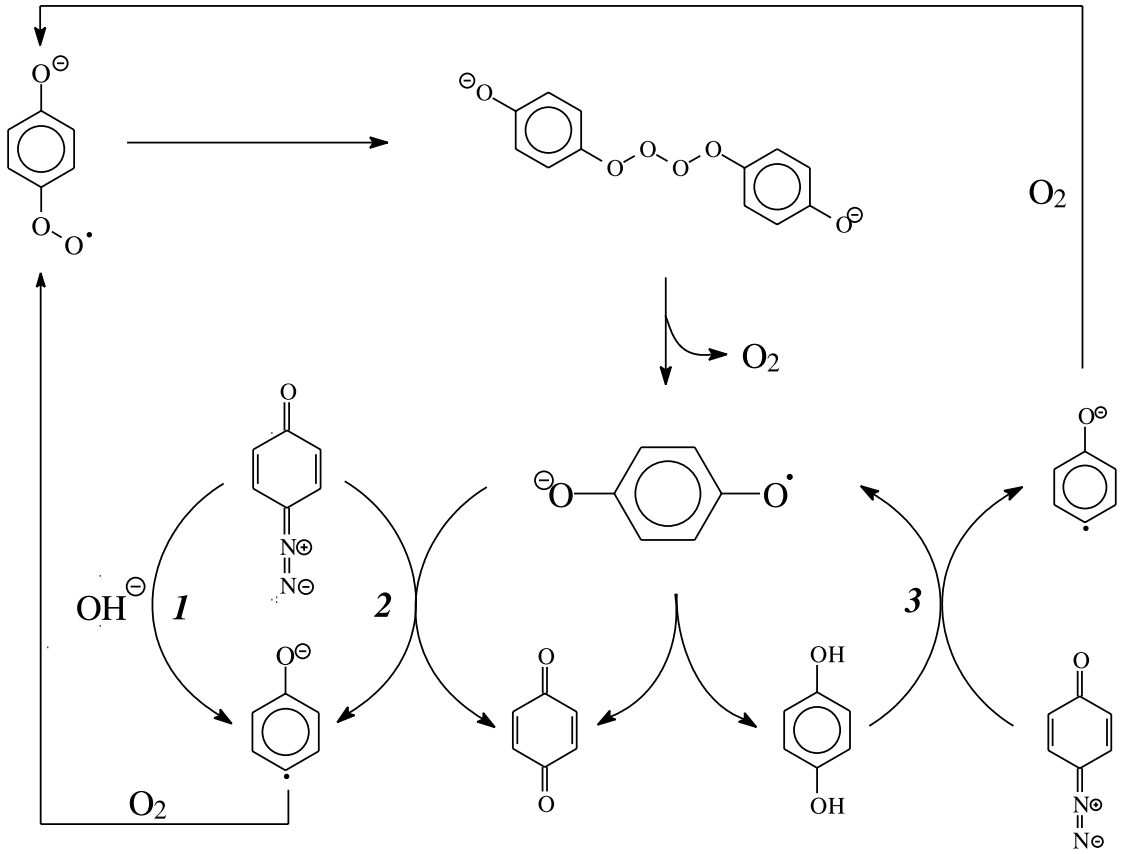
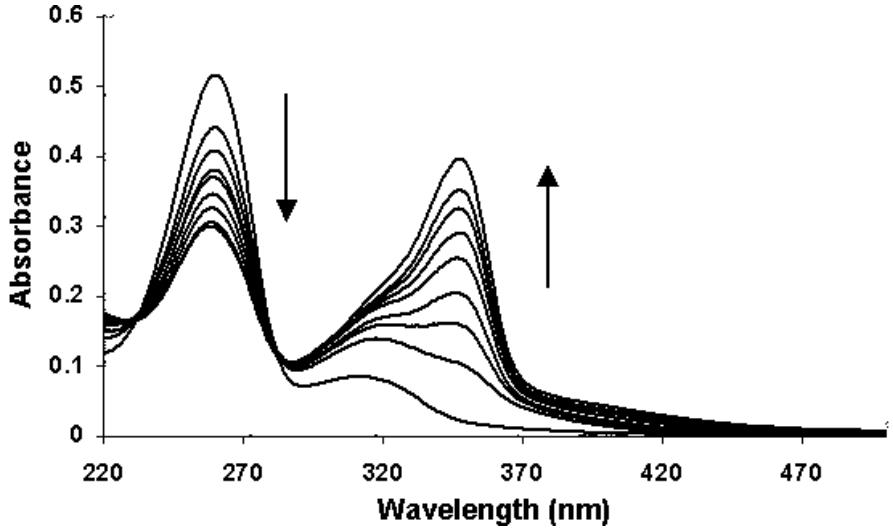


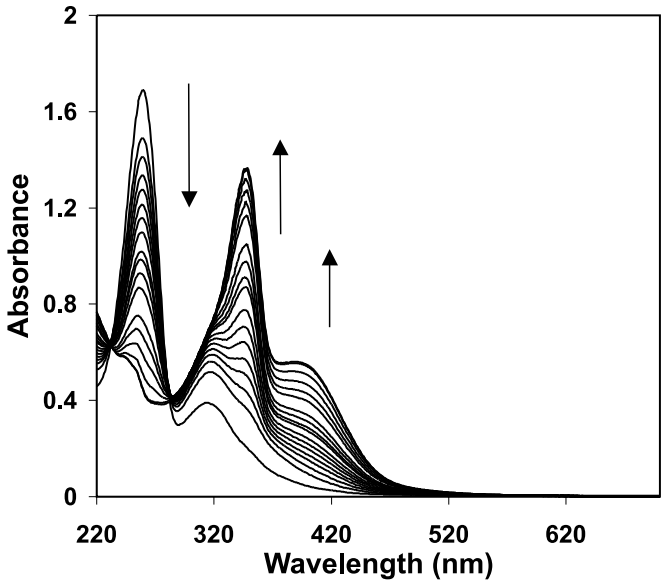

**Fig. 1.** Absorption spectra measured at different times (total time: 2 h) with $3.26 \times 10^{-5}$ M aliquots taken from 1.36 mM pNO buffered solutions (phosphate buffer, pH 7.2, 0.1 M, treated with Chelex 100) kept in darkness at 37°C.




data published in the literature (9–11), and once again we found an NB peak. Since no hydrogen-atom donor could be present under our experimental conditions we are currently investigating the possible interference of an instrumental artifact.

The lack of reactivity of pNO to thermal solvolytic decomposition and the subsequent formation of the aryl ion has been reported elsewhere (11), where it has been put down to the propensity of the nitro substituent to destabilize the aryl cation more than it does the arenediazonium ion (11, 19). In addition to this we checked the photochemical stability of pNO during our experiments by comparing the spectrum of a sample irradiated in a quartz UV spectrophotometer cuvette under the experimental conditions routinely used throughout the spectrophotometric analysis with that of an aliquot kept in darkness at the same temperature. These data

**Fig. 2.** Absorption spectra measured at different times (total time: 4 h 40 min) with a 0.11 mM pNO buffered solution (phosphate buffer, pH 7.2, 0.1 M, treated with Chelex 100) kept at 37°C.




led us to conclude that the formation of an aryl cation from the thermal and (or) photochemical heterolytic decomposition of pNO should not be expected. Therefore, we have initially considered that the decomposition of both benzenediazonium ions can be described according to Scheme 2.

In this scheme it is accepted, according to the well known Gomberg–Bachman reaction and the mechanism described by Rüchardt and Merz (20), that in the absence of any other reductant the dediazoniation process for both benzenediazonium ions has a common starting point, the attack of the hydroxide anion (reaction **a**) to form a covalently bound intermediate, diazohydroxide, in equilibrium with its dissociated form, diazotate (reaction **b**).

In principle, two parallel pathways (Scheme 2), both mediated by the diazotate anion, would explain the products derived from the dediazoniation of PDQ and pNO. Thus, $-OArN_2^+$ might be formed via aromatic nucleophilic displacement. Clearly, this process would only be detectable with pNO since with PDQ the reactant ($RArN_2^+$, where R = $^-$O at the pH of the medium) and reaction product ($-OArN_2^+$) are identical. Whilst a hydroxide ion may act as the nucleophile to displace the nitrite ion from the initial arenediazonium ion, Kuplet-skaya and Kazitsyna (21) have also described such a substitution involving the diazotate ion as the nucleophile. Here an attack by $O_2NArN = N\text{-}O^-$ on pNO followed by the displacement of $NO_2^-$ would give a diazotate ether, $O_2NArN = N\text{-}O\text{-}ArN_2^+$; heterolytic dissociation of this ether regenerates pNO and gives the $^-OArN_2^+$ product. Thus we checked whether a kinetic analysis could confirm this bimolecular process. The nucleophilic attack by the diazotate anion can be expressed by the following set of equations:

$$[1] \qquad \frac{-\mathrm{d[pNO]}}{\mathrm{d}t} = \frac{\mathrm{d[PDQ]}}{\mathrm{d}t} = k[\text{diazotate}][\text{pNO}]$$

$$K_\mathrm{b} = \frac{[\text{diazotate}][\mathrm{H^+}]}{[\text{diazohydroxide}]} K_\mathrm{a} = \frac{[\text{diazohydroxide}]}{[\text{pNO}][\mathrm{OH^-}]}$$

$$[2] \qquad \frac{-\mathrm{d[pNO]}}{\mathrm{d}t} = kK_\mathrm{a}K_\mathrm{b}\frac{[\mathrm{OH^-}]}{[\mathrm{H^+}]}[\text{pNO}]^2 = k_\mathrm{g}[\text{pNO}]^2$$

in which the rate of PDQ formation is equal to the pNO decomposition rate (eq. [1]), in accordance with the spectroscopic results shown in Fig. 1, where it is apparent that very little interference from pNP should be expected. We tested the final equation (eq. [2]) with the absorbance values measured at 350 nm and thus obtained a plot with a value of 88.9 $M^{-1}\cdot min^{-1}$ for the global constant $k_g$ (Fig. 3). This value, which is significantly lower than that obtained for the reduction of pNO with hydroquinone (22), suggests the absence of any reductant capable of directly reducing pNO by a "non-bonded" outer-sphere mechanism. Besides this, the kinetic results support a bimolecular reaction, involving diazotate anion as a nucleophile, since no better fitting of the experimental data was obtained by considering a first-order reaction, which might be expected as a result of a nucleophilic substitution by $OH^-$.

The other product (RArOH) formed from the dediazoniation of PDQ and pNO might be the result of a reaction between the diazotate anion and the diazonium ion to give diazoanhydride, which then breaks down homolytically to produce the diazenyl radical, from whence is formed the aryl radical, and a further reaction with molecular oxygen leads to a substituted phenol, as indicated for PDQ in Scheme 1. Nevertheless, such a mechanism is not supported by the concentration dependence observed in the proportion of the major products (PDQ and pNP) derived from the decomposition of pNO. If pNP and PDQ were the products that occurred as a result of a parallel reaction during pNO decomposition, an increase in the concentration of pNO would not justify an increase in PDQ concomitant with a decrease in pNP, as can be observed by comparing Figs. 1 and 2. Furthermore, the formation of hydroquinone from PDQ occurs as a result of a homolytic pathway that involves molecular oxygen (Scheme 1), whereas pNP appears when either aerated or deaerated samples of pNO are incubated. An alternative pathway for pNP formation can be formulated on the basis of the homolytic breakdown of diazohydroxide, which leads to the appearance of the aryl and hydroxyl radicals. In fact it has been pointed out (16) that pNO is particularly reactive in neutral buffers, giving EPR signals corresponding to adducts of •OH and aryl radicals even without any added electron donors. A few years ago some discussion arose as to whether a hydroxyl radical might not be involved in the dediazoniation of benzenediazonium ions. Thus •OH adducts detected by EPR have been considered to be artifacts resulting from the fragmentation of a transient hydroxylamine (16). On the other hand, the results obtained by Lawson, Gannett, and co-workers (23, 24) under conditions where no reducing agent was present appear to be consistent with the homolytic fragmentation of a diazohydroxide intermediate. In the light of this latter experimental evidence, therefore, we believe that the recombination of aryl and hydroxyl radicals would provide a simple explanation for the appearance of pNP.

Thus, on the basis of the experimental results obtained, a tentative general mechanism can be proposed, as described in Scheme 3.

**Scheme 2.**

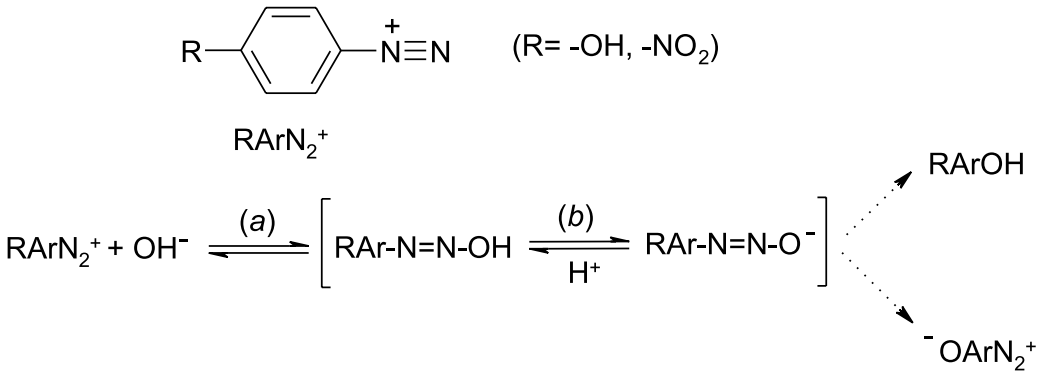
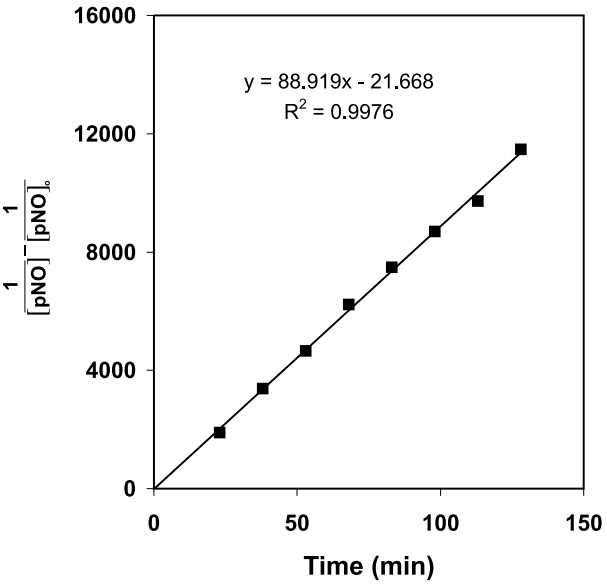



**Fig. 3.** Kinetic analyses of the data obtained from the absorbance measured at 350 nm in the spectra shown in Fig. 1.




It is clear that under our experimental conditions PDQ dediazoniation occurs mainly via the reaction sequence (**a**) → (**b**) → (**f**) → (**h**) → (**i**), wherein reaction **i** encompasses the processes detailed in Scheme 1, producing hydroquinone–quinone as stable products of the reaction and secondary reductant (hydroquinone), whereas pNO decomposition might occur either via (**a**) → (**b**) → (**e**) → (**g**), which generates PDQ, or the homolytic path (**a**) → (**c**) → (**d**), which produces pNP.

The differences observed in the dediazoniation mechanisms for PDQ and pNO might be put down to the different chemical natures of the aromatic substituents in the compounds analysed. The electron-withdrawing and donating properties of the $-NO_2$ and -OH groups strongly determine the reactivity of either compound and consequently exert different influences upon the dediazoniation reaction, as observed experimentally.

## DTPA interference into PDQ and pNO dediazoniation

PDQ dediazoniation in a neutral aqueous medium was clearly affected by the presence of DTPA either using a phosphate buffer treated with Chelex resin or an untreated phosphate buffer. No change in the spectrum shape was noticeable following the addition of DTPA, suggesting that any interaction between DTPA and PDQ to block the reduction of this latter compound might be ruled out. Apart from this, the introduction of DTPA led to a period of about 2 h during which the reaction seemed to cease. After this period decomposition continued at a rate very close to that observed for the reaction in the absence of DTPA (Fig. 4). Moreover, the presence of DTPA appears to be associated with a decrease in oxygen consumption, as shown in Fig. 5.

On the other hand, the dediazoniation of pNO behaved differently in the presence of DTPA, its dediazoniation rate increasing (Table 1). Likewise, DTPA brought about an increase in the proportion of pNP in relation to PDQ (cf. Table 1 and Fig. 6). Furthermore, pNO underwent complete and immediate decomposition when the initial DTPA:pNO ratio was 5 or higher. In this case only two absorption bands were recorded, with maxima at 270 nm and 369–370 nm. Similar results (Fig. 7) were obtained by adding ethanol (4%) to the buffered solutions of pNO (maxima at 268 and 388–390 nm).

If we exclude any association between the arenediazonium ions and DTPA or chelating effects upon any residual metal ions present in the buffer, an additional pathway must be considered to justify the observed increase in the decomposition rate, wherein DTPA would promote the homolysis of pNO. As a matter of fact, on using a higher concentration of DTPA (6.6 mM) this additional pathway plays a more important part, as indicated by the appearance of an absorption band with a maximum at about 270 nm. This band also appears when pNO decomposes in the presence of ethanol (4%), which may scavenge aryl radicals by donating a hydrogen atom (11). These spectrophotometric results are consistent with HPLC analyses, which showed, together with the peaks corresponding to PDQ and pNP, the competitive formation of an appreciable quantity of NB, resulting in the almost complete conversion of pNO into NB when the ethanol concentration was increased to 50%.

Bearing in mind the fast rate of dediazoniation in the presence of a relatively high concentration of DTPA and also the appearance of NB under such conditions, it is reasonable to suppose that DTPA triggers the homolytic decomposition of pNO. Some experimental evidence is reported in the literature concerning the scavenging capacity of DTPA to react with •OH and $CO_3^{\bullet -}$ when used in relatively high concentrations (>100 μM) (25). The data obtained during pNO dediazoniation suggest the possible formation of a DTPA

**Scheme 3.**

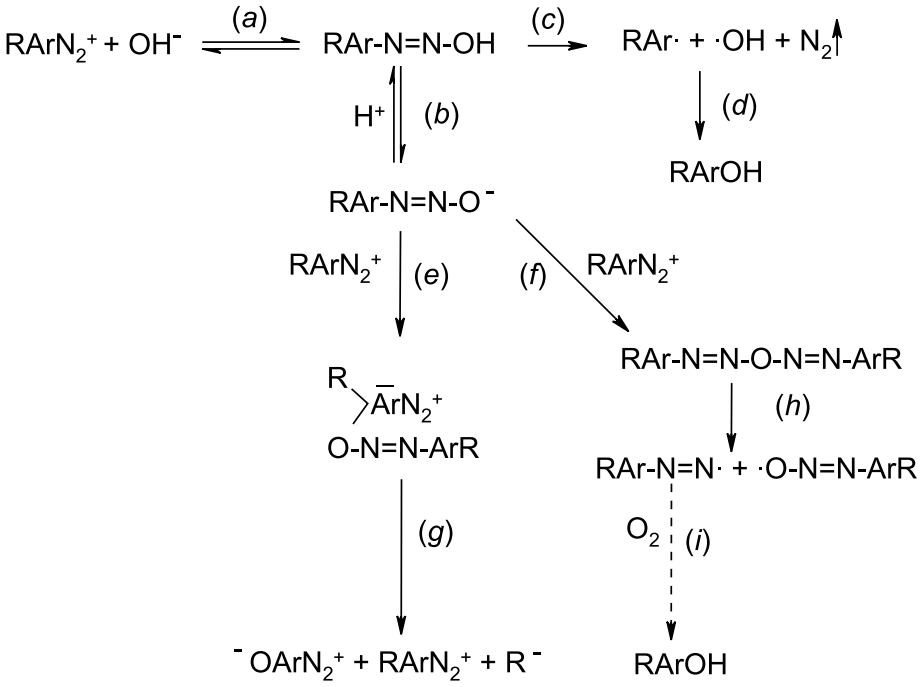



**Fig. 4.** Plot of the absorbance measured at 348 nm vs. time obtained with 1:40 diluted aliquots taken from 0.4 mM PDQ buffered solutions (phosphate buffer pH 7.2; 0.1 M) kept in darkness at 37°C. Influence of the DTPA concentration: (*a*) without DTPA; (*b*) in the presence of 1 mM DTPA; and (*c*) in the presence of 1.5 mM DTPA.

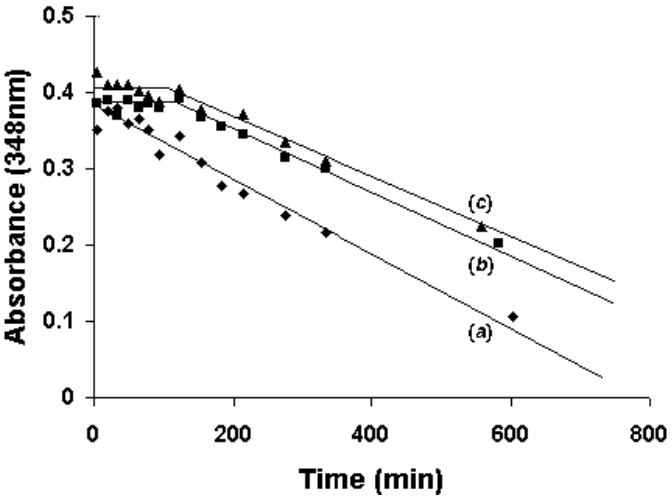
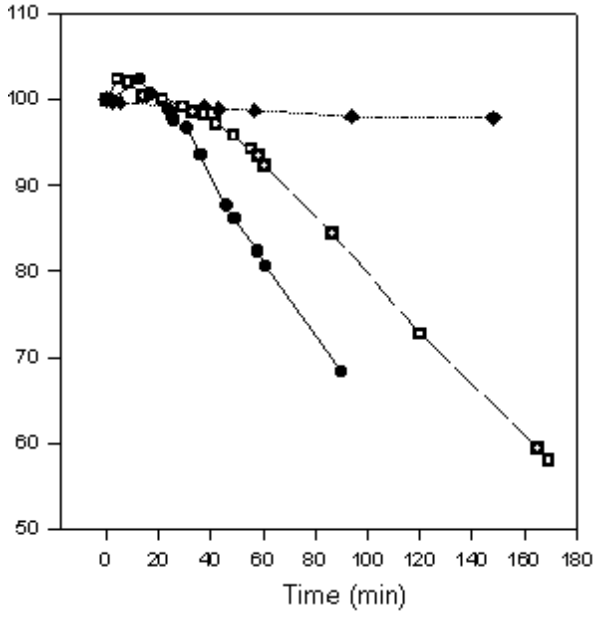



**Fig 5.** Plot of oxygen consumption vs. time for samples containing distilled water (◆), 10 mM PDQ in the presence of 0.5 mM DTPA in phosphate buffer pH 7 (□), and 10 mM PDQ solution in phosphate buffer pH 7 (●). *T* = 37°C.




radical that interacts with pNO, thus increasing the decomposition rate according to the following general scheme:

[3] pNO → $O_2NArN_2$•

[4] $O_2NArN_2$• → $O_2NAr$• + $N_2$

[5] $O_2NAr$• + DTPA → NB + [DTPA]•

[6] pNO + [DTPA]• → $O_2NArN_2$• + DTPA(H+)

wherein reaction [3] summarizes pathways (**a**) → (**b**) → (**f**) → (**h**) whilst reactions [3] and [4] are equivalent to the pathway (**a**) → (**c**). In any case the occurrence of the aryl radical $O_2NAr$•, derived from pNO (11), allows the interfering DTPA scavenging aryl radical to produce NB (reaction [5]) and also leads to the subsequent attack on pNO (reaction [6]), which could go on to give pNP via pathway (**i**).

It is apparent that these reactions do not work in the case of PDQ because an increase in the concentration of DTPA is not followed by a concomitant increase in the decomposition of PDQ, as observed for pNO. Nevertheless, a similar reac-

**Table 1.** Influence of DTPA upon pNO (0.11 mM) decomposition rate at 37°C in phospate buffer (pH = 7.2).[a]

| [DTPA] ($mol \cdot L^{-1}$) | Total time (min) | [PDQ] ($mol \cdot L^{-1}$ (%)) | $[pNP]_T$[b] ($mol \cdot L^{-1}$ (%)) | [pNO][c] ($mol \cdot L^{-1}$ (%)) |
|---|---|---|---|---|
| 0 | 280 | $2.5 \times 10^{-5}$ (23) | $6.7 \times 10^{-5}$ (62) | $1.6 \times 10^{-5}$ (15) |
| $1 \times 10^{-4}$ | 99 | $0.9 \times 10^{-5}$ (9) | $7.8 \times 10^{-5}$ (77) | $1.4 \times 10^{-5}$ (14) |
| $2 \times 10^{-4}$ | 45 | $0.6 \times 10^{-5}$ (6) | $8.3 \times 10^{-5}$ (81) | $1.3 \times 10^{-5}$ (13) |

[a]Analyses carried out by monitoring absorbance at 259, 350, and 400 nm and using the following ε ($L \cdot mol^{-1} \cdot cm^{-1}$) values: 2500 (dissociated pNP, 259 nm); 1320 (undissociated pNP, 259 nm); 5230 (dissociated pNP, 350 nm); 3950 (undissociated pNP, 350 nm); 15 480 (dissociated pNP, 400 nm); 70 (undissociated pNP, 400 nm); 3050 (PDQ, 259 nm); 41 990 (PDQ, 350 nm); 15 590 (pNO, 259 nm); 325 (pNO, 350 nm). $pK_a$ = 7.15 for pNP at 25°C was taken from ref. 26.

[b]$[pNP]_T$ = total concentration of *p*-nitrophenol.

[c]pNP and PDQ are taken to be the main products derived from the decomposition of pNO.

**Fig. 6.** Absorption spectra measured at different times (total time: 1 h 39 min) with a 0.11 mM pNO buffered solution (phosphate buffer, pH 7.2, 0.1 M, treated with Chelex 100) kept at 37°C in the presence of 0.1 mM DTPA.

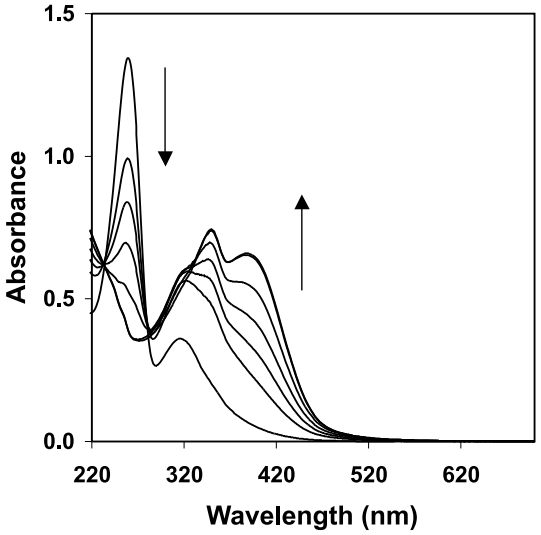
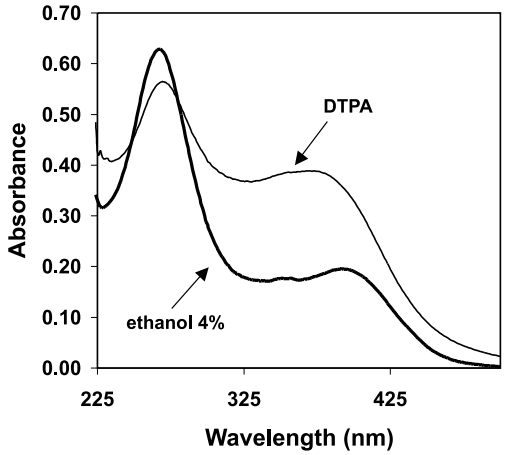
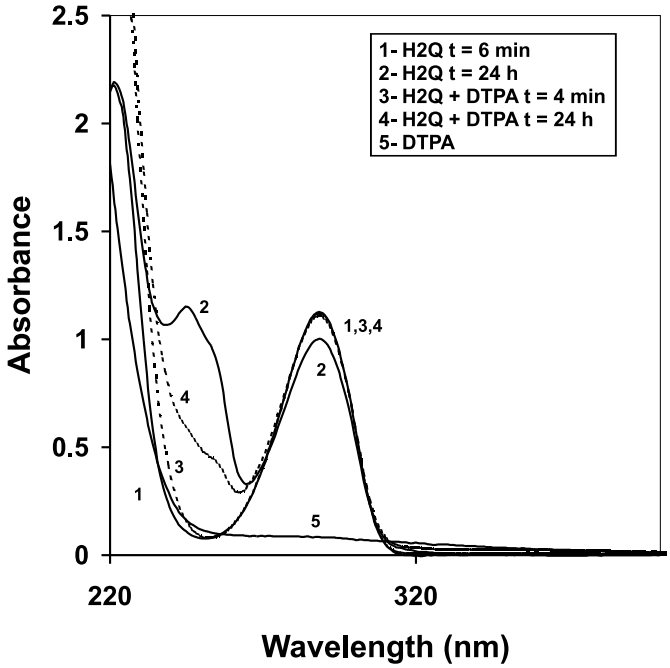



**Fig. 7.** Absorption spectra measured at 5 min with 1:10 diluted aliquots taken from 1.33 mM pNO buffered solutions (phosphate buffer, pH 7.2, 0.1 M, treated with Chelex 100) kept at 37°C in the presence of (*a*) 6.65 mM DTPA; (*b*) ethanol 4% (v/v).




tion pattern might occur with PDQ in the presence of DTPA, although not affecting PDQ directly. In fact we have observed that the absorption spectrum of a 0.44 mM hydroquinone (H2Q) solution in phosphate buffer at pH 7 changes

**Fig. 8.** Absorption spectra measured at different times with 0.44 mM hydroquinone (H2Q) buffered solutions (phosphate buffer pH 7) both in the presence and absence of DTPA (2.8 mM). *T* = 37°C. See legends in the inset to identify the spectra.




24 h after the solution is prepared. The band located at 288 nm decreases (approx. 11%), and a new band appears centred at 245 nm. In the presence of DTPA (2.8 mM) the band at 288 nm remains practically constant, and the band at 245 nm is significantly less intense (Fig. 8). These results indicate that a relatively high concentration of DTPA can lead to an interference that affects the intermediate semiquinone radical in hydroquinone auto-oxidation. Bearing this in mind, the decrease observed in the PDQ decomposition rate would be justified since PDQ reduction by the semiquinone scavenger radical would be partially interrupted, thus giving rise to a concomitant decrease in oxygen consumption.

## Conclusion

In summary, we have found that the chelating agent DTPA interferes with the dediazoniation of *p*-hydroxybenzenediazonium and *p*-nitrobenzenediazonium ions. Although the different pattern of decomposition observed for both ions,

either in the absence or presence of DTPA, suggests the existence of different mechanisms for each, most of our observations strongly support the idea that this interference may be because of a common mechanism based on the scavenging activity of DTPA. Therefore any possible interference by DTPA should be thoroughly investigated when it is used as a chelator in biochemical and biological systems in which radical reactions occur.

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