Reactions of orthophthalaldehyde with ammonia and 2-aminoethanol†

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Reactions of orthophthalaldehyde (OPA) with amines are used in the determination of amino acids and in applications of OPA as a biocide. To contribute to the understanding of processes involved, the reactions of OPA with ammonia, which are conveniently slow, were studied. In a set of rapidly established equilibria, the 1,3-dihydroxyindole and the product of its dehydration are formed (Scheme 1). The individual equilibria were identified and equilibrium constants determined using DC polarography and UV spectra. The ring closure involves the carbinolamine; the imine formation is a side reaction. Both the ring formation and the dehydration of the carbinolamine are generally acid catalyzed. In the finally established overall equilibria between OPA and the isoindole derivative, the concentrations of intermediates are negligible. The same applies to the reaction of OPA with 2-aminoethanol, in which the initial formation of a carbinolamine and of an imine are too fast to be followed. Very slow reactions taking place during periods of hours or days, which probably result in the formation of dimeric species, have also been observed. This contribution demonstrates the advantages of combinations of polarographic and spectrophotometric techniques in the investigation of complex reactions of some organic compounds.

Introduction

Roth[1] followed the fluorescence of a product of the reaction of amino acids with orthophthalaldehyde. He observed that the intensity of fluorescence increases in the presence of a strong nucleophile, which has no tendency to a ring formation, such as thiolate. He also reported[1] that the intensity of the fluorescence was higher when the sample of the amino acid was added to the reaction mixture after the addition of the strong nucleophile to the solution of OPA, than when the sequence of additions was reversed. A similar difference was observed for the determination of ammonia and other primary amines[2] in the presence of thiols and other strong nucleophiles, such as recently preferred cyanide ions.[3] The sequence of the addition was followed in the several hundred reports dealing with determination of amino acids,[3] but no attempt has been made to propose a plausible explanation for the procedures used.

The conditions for analytical procedures were established empirically. As a result, the reported recommended conditions for individual analyses widely differ[4] and the reproducibility in different laboratories is questionable. The situation does not fulfil the condition for reliable analytical methods, namely that the nature of physical and chemical processes involved should be understood at least to some degree. The same applies to the applications of OPA as a biocide, used, for example, in disinfection of surgical instruments.

Attempts to describe the sequence of reaction steps involved[3] were based on the assumption that OPA is present in the cyclic hemiacetal form, and on an identification of the fluorescent product. Proposed reaction schemes involved implausible steps, such as fast dissociation of C-acids. To contribute to a better understanding of reactions of OPA in aqueous solutions, the equilibria involving OPA in such solutions were first investigated.[4]

At pH > 10.5, addition of hydroxide ions to a formyl group in OPA takes place. The resulting geminal diol anion can undergo electro-oxidation.[4]

In the course of a gradual elucidation of the sequence of chemical reactions taking place in aqueous solutions containing OPA, in this contribution were investigated the reactions of OPA with ammonia and 2-aminoethanol (colamine, RNH_2). The latter was chosen because it is a non-volatile, water soluble amine with a pK_a 9.5, close to that of amino acids, but without the complicating acid–base properties of the carboxylic group. The investigation of reactions of OPA with amino acids and nucleophiles, such as CN^- and thiolate ions is in progress.

To investigate the establishments of equilibria in solutions containing OPA and NH_3 or colamine and in some cases of the kinetics of some of the involved reactions, the use of polarography (recording of current–voltage curves using a dropping mercury electrode) proved useful. The use of this technique for determination of equilibrium constants of reactions of various carbonyl compounds with primary amines was reported early.[5] The decrease of polarographic waves of OPA in the presence of amino acids and ammonia was used early for analytical purposes,[6] but the nature of the processes involved was not elucidated.

In some instances, particularly when following kinetics of fast initial reactions, spectrophotometry was used. The reasons for the preference of the one or the other technique will be discussed below.

The combination of chemical reactions involved and of electroreductions of the starting material **1** (1,2-benzenedicarboxaldehyde), of side reaction products **2** (2-formylbenzaldehyde hydrate) and **3**, (1,3-dihydroxyisobenzofuran) of the reaction intermediates **5**, (2-methylol-1-benzcarboxaldehyde imine **6**,

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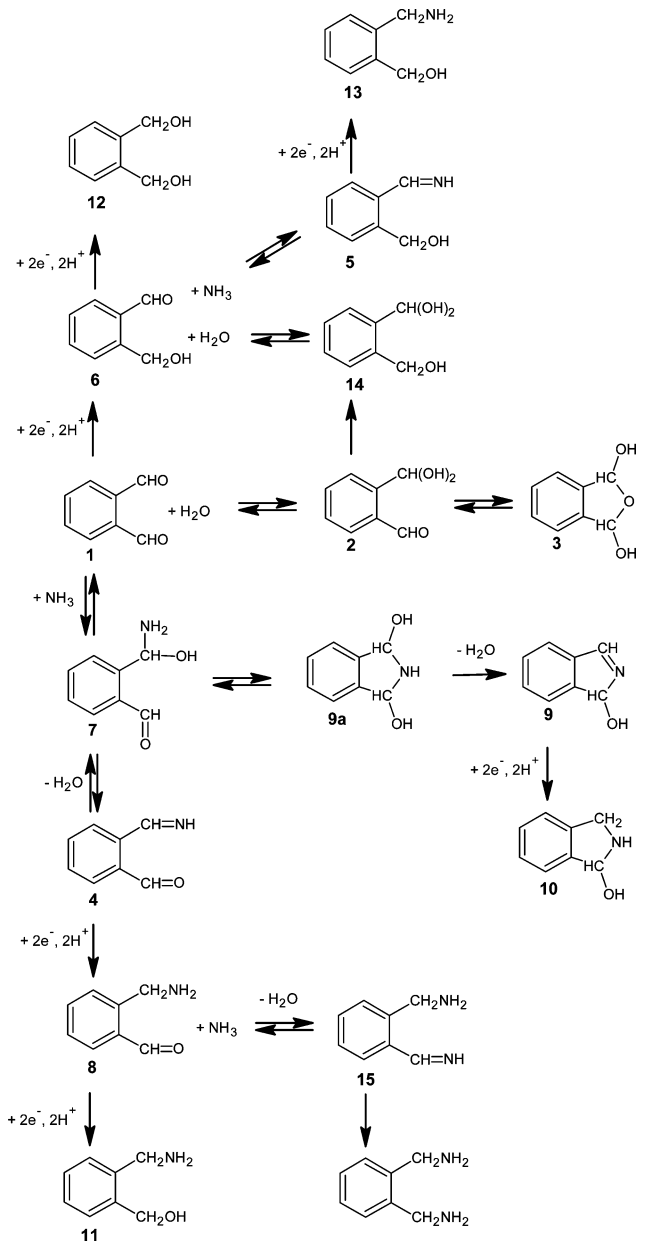
(2-methylol-1-benxenecarboxaldehyde) **7**, (1′-hydroxy-1′amino-2-formyl-toluene) **9**, (1-hydroxy-isoindoline) and **14**, (3-methylol-benzenecarboxaldehyde hydrate) as well as of the reduction intermediates **4** (2-formyl-benzenecarboxaldehyde imine), **6**, (2-methylol-benzenecarboxaldehyde imine) and **8**, (2-formyl 1′-aminotoluene) of the final reaction product **9** (1-hydroxy-isoindoline **11**, (2-aminomethyl-1′-hydroxytoluene **12**, (2-methylol-1′-hydroxytoluene) and **13** (2-methylol-1′-aminotoluene) are depicted in Scheme 1.



Scheme 1 Reaction of orthophthalaldehyde (OPA) with ammonia (NH_3).

Results and discussion

Equilibria in the reactions of orthophthalaldehyde (OPA) with ammonia

OPA is reduced in the investigated pH-range in the absence of ammonia in borate or 4-hydroxybenzenesulfonate buffers in two polarographic waves, i_1 and i_2. The more positive wave i_1 [which at pH 9.3 has a half-wave potential of –0.88 V (SCE)] corresponds to a reduction of the unhydrated form of OPA **1**. In the second, more negative wave i_2, which in these buffers has $E_{1/2} = -1.3$ V, the aldehydic group in the monohydrated, acyclic form of OPA **2** undergoes reduction. In the pH range between pH 8.3 and 10.5, in which this investigation was carried out, the limiting currents i_1 and i_2 are controlled by the ring opening of the cyclic hemiacetal form **3** and by the rate of dehydration of form **2**, which are base catalyzed.[4] Due to such catalysis, the equilibria between forms **1**, **2**, and **3** of OPA are established rapidly, when compared to the rate of establishment of equilibria between the form **1** of OPA and ammonia.

Currents i_1 and i_2 remain a linear function of concentrations of forms **1** and **2**. Due to the relatively rapid establishment of equilibria between forms **1**, **2**, and **3**, the current i_1 remains in the reaction with NH_3 a linear function of the concentration of the unreacted OPA. The majority of investigations of the equilibria in the reactions of OPA with NH_3 were carried out at pH 9.3, at equal concentrations of NH_3 and NH_4Cl. These solutions acted simultaneously as buffers, as a reagent and as a supporting electrolyte. To keep a sufficient excess of the supporting electrolyte at $[NH_4^+] < 0.1$ M, the ionic strength was kept at $\mu = 0.1$ by addition of potassium chloride.

With increasing concentration of the ammonia–ammonium chloride buffer pH 9.3, waves i_1 and i_2 decrease and wave i_3 (Fig. 1) increases. The decreases of waves i_1 and i_2 are due to the reaction of OPA with NH_3 yielding the carbinolamine **7**. This compound **7** will be predominantly formed by addition of NH_3 to the unhydrated

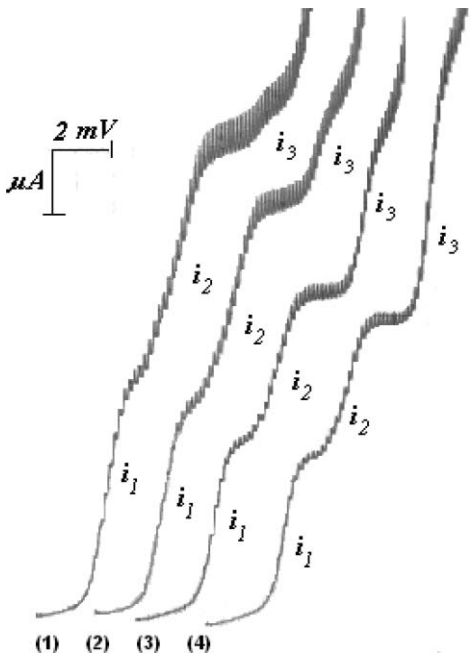


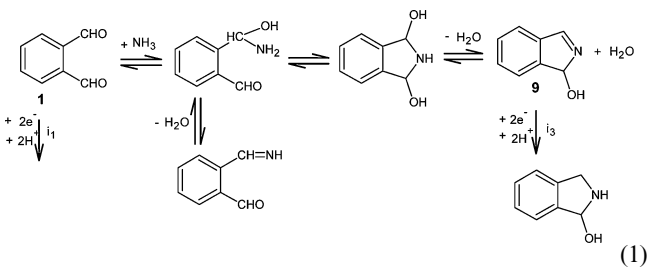
Fig. 1 Dependence of polarographic current–voltage curves in solutions of 1×10^{-4} M orthophthalaldehyde on concentration of a solution of (1) 0.1 M NH_3 and 0.1 M NH_4Cl, (2) 0.5 M NH_3 and 0.5 M NH_4Cl, (3) 0.6 M NH_3 and 0.6 M NH_4Cl, and (4) 0.8 M NH_3 and 0.8 M NH_4Cl. Curves starting at –0.6 V approximately 30 min after mixing. Waves i_1 and i_2 are due to the reduction of the starting dialdehyde, waves i_3 to the product—dehydrated 1,3-dihydroxyisoindole.

form of OPA **1**. When the equilibrium between forms **1** and **2** is perturbed by the reaction of **1** with NH_3, further unhydrated form **1** can be generated from forms **2** and **3**, as the equilibria between forms **1**, **2** and **3** are established rapidly, compared to the equilibrium of the reaction, in which form **1** is converted into the carbinolamine **7**. At higher concentrations of NH_3, it is not possible to exclude that some of the carbinolamine **7** is formed by addition of NH_3 to monohydrated acyclic form **2** followed by a fast dehydration of the geminal diol group.

It is the carbinolamine that then forms the cyclic intermediate **9a**. In the initial stages of the reaction between OPA and NH_3 (present in concentrations lower than 0.1 M), a formation of the wave i_1' at potentials more positive than wave i_1 takes place. This wave is attributed to the reduction of the imine **4**, facilitated by the formyl group in the *ortho*-position. A similar easier reduction of an imine, when compared to the parent carbonyl compound, has been observed for other substituted benzaldehydes.[5] Similarly, wave i_2' is attributed to the reductions of imines **5** and **15**, bearing in the *ortho*-position a CH_2OH **5** or CH_2NH_2 **15** group. Their reductions occur at more positive potentials ($E_{1/2} = -1.13$ V) than those of parent benzaldehydes **6** and **8**, which are reduced in a wave i_2 ($E_{1/2} = -1.3$ V). The waves of the monohydrated form **2** and of the 2-methylolbenzaldehyde **6** are not separated due to the similarity of the substituent effects of 2-CH_2OH and 2-$CH(OH)_2$. The monoimine **4** is in this case the product of a side reaction, because the carbinolamine **7** undergoes a competitive rapid cyclization into **9a** (Scheme 1).

A decrease in wave i_1, with increasing concentration of NH_3 in the used buffer, is accompanied by an increase of wave i_3 at more negative potentials [$E_{1/2} = -1.6$ V (SCE)] (Fig. 1). The potential range, in which wave i_3 is reduced, indicates the presence of a species in which the benzene ring is conjugated with a double bond. Primary cyclization product **9a** does not contain a reducible double bond. A reductive cleavage of one of the C–OH bonds does not seem to be sufficiently activated.

When the reaction of OPA with amines was carried out in nonaqueous solvent, such as DMSO or acetonitrile,[7] it yielded a phthalimidine. This compound was prepared and found not to be reducible at pH 9–11 at potentials more positive than –1.9 V. As a working hypothesis, it is assumed that the species **9a** undergoes dehydration in aqueous solutions and the resulting isoindole derivative **9** is the compound that undergoes two-electron reduction in wave i_3, following eqn (1).



(1)

At pH 8.3–10.3 at constant [NH_3] and varied [NH_4^+], the height of wave i_1 and the ratio of $i_1 : i_3$ remained pH-independent. The overall equilibrium is thus not affected by pH, as can be expected. Hence the overall equilibrium of the sequence in eqn (1) can be followed by a determination of the ratio $i_1 : i_3$.

When currents i_1 and i_3 were plotted as a function of log[NH_3] after establishment of the overall equilibrium (Fig. 2), the plot for i_1 had the shape of a decreasing dissociation curve, that of $i_3 = f(\log[NH_3])$ of an increasing dissociation curve. The inflection point of such a dissociation curve corresponds to an equilibrium constant $K_{overall} = [\mathbf{9}]/[\mathbf{1}]\,[NH_3]$ for the equilibrium between forms **1** and **9**. This equilibrium constant has a value $K_{overall} = 4 \times 10^2$ L mol^{-1}. For comparison, the equilibrium constant for the reaction of isophthalaldehyde with ammonia was found[8] to be 2.5×10^{-1} L mol^{-1} and that for the reaction of terephthalaldehyde with ammonia was reported[8] to be 4.75×10^{-1} L mol^{-1}. This indicates the possibility of the strong role of cyclization on the reactivity of 1,2-benzenedicarboxaldehyde in the reaction with ammonia.

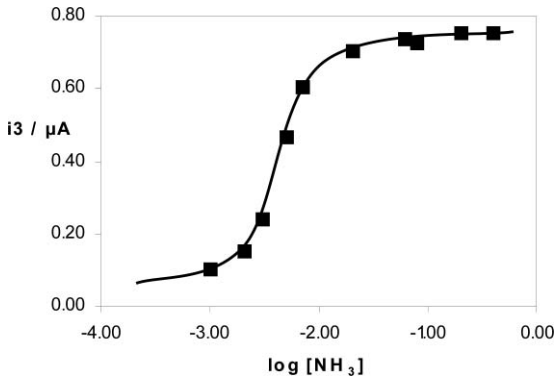


Fig. 2 The dependence of limiting currents, (i_3), at equilibrium for the slower reaction in a solution of 1×10^{-4} M orthophthalaldehyde in a pH 9.3 ammonia buffer composed of different ratios of [NH_3] : [NH_4Cl], and keeping the ionic strength constant with KCl.

The fit of experimental data to the theoretical dissociation curve for i_3 in Fig. 2 represents a proof of establishment of an overall equilibrium in the reaction of OPA with NH_3. In this equilibrium, the unhydrated form **1** of OPA, which is reduced in wave i_1 at –0.9 V at pH 9.3, yields the isoindole derivative **9**, which is reduced in wave i_3 at –1.6 V.

To offer further support of the reversibility of all the processes given in eqn (1), the reaction mixture, in which the species **9** was formed and equilibrium was established, was acidified by addition of acetic acid to a pH between 4.2 and 4.8. Nevertheless, the *i*–*E* curves obtained after acidification differed from that of the parent OPA. In the presence of NH_4^+ as a proton donor, an acid catalyzed reaction takes place in the vicinity of the electrode surface. The product of this reaction is a benzaldehyde derivative, reduced in a single wave at about –1.1 V. The nature of this reaction was not further investigated and polarography proved not to be a technique suitable for testing the reversibility of this reaction.

The sought support was obtained by using spectrophotometry. When the solution, in which the product **9** was formed at pH 9.3, was acidified to pH 4.2 to 4.8 by addition of acetic acid within about 30 min after the mixing of solutions of OPA and NH_3 at pH 9.3, the characteristic absorption bands of species **9** at 250 and 284 nm in the spectra disappeared and those of OPA at 240 and 260 nm appeared. This proves the establishment of all equilibria in reaction (1). Spectra recorded immediately after acidification decreased with time until a hydration–dehydration equilibrium was established. The form **1**, generated by the hydrolysis of species

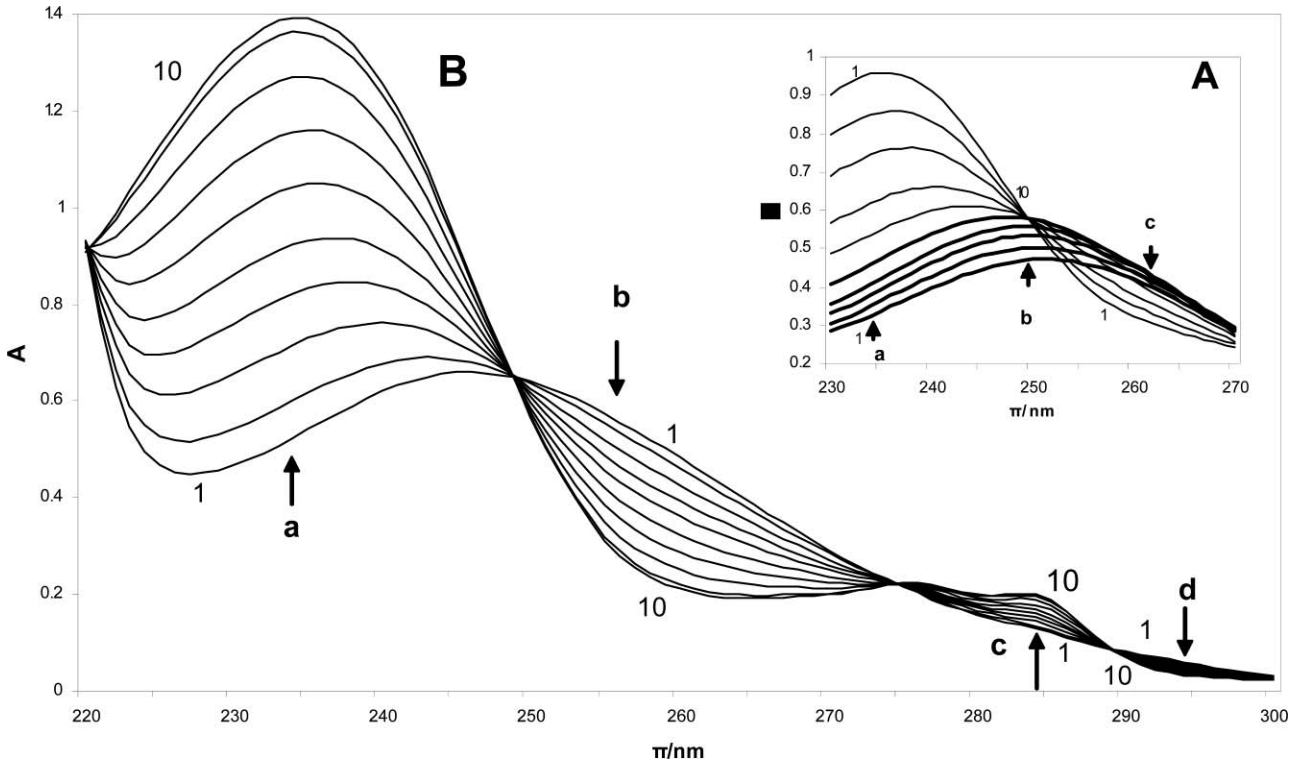


Fig. 3 **A** The dependence of spectra for the fast reaction followed by the slower reaction in a solution of 1×10^{-4} M orthophthalaldehyde in a buffer composed of 0.1 M NH_3, 0.1 M NH_4Cl and 2.9 M KCl at pH 9.3. Spectra 1–10 correspond to times: (1) 0.5, (2) 0.7, (3) 1.02, (4) 1.47, (5) 3.23, (6) 7.65, (7) 12.72, (8) 21.68, (9) 30.88 and (10) 42.58 min. (a) Product, (b) intermediate imine in a side reaction, (c) starting material. **B**. The slower reaction of 1×10^{-4} M orthophthalaldehyde in a buffer composed of 1 M NH_3 and 1 M NH_4Cl at pH 9.3. Spectra recorded after (1) 0.58, (2) 1.07, (3) 1.88, (4) 2.77, (5) 3.77, (6) 5.22, (7) 7.17, (8) 10.25, (9) 16.55 and (10) 25.15 min. Spectra (a, c) of the product and (b, d) of the starting material. Three isosbestic points.

9, is thus converted into forms **2** and **3** and new equilibria are established.‡

When the reaction mixture after establishment of an equilibrium was kept for more than 1 h before acidification, the changes of absorbances with time did not result in the original spectra of OPA. This is due to very slow consecutive reactions discussed below.

Kinetics of reaction of OPA with ammonia

In the reaction of orthophthalaldehyde (OPA) with ammonia, it was possible to distinguish three phases: (a) a fast reaction that at 25 °C plays a role only over the first two minutes. (b) A slower reaction can be followed over periods between 5 and 60 min. (c) At time-periods longer than about 60 min, very slow reactions, yielding products absorbing at wavelengths longer than 350 nm and yielding fluorescent species, were observed.

Kinetics of the fast reaction

At times shorter than about 120 s in solutions of OPA at pH 9.3 containing NH_3 at a concentration lower than 0.1 M, an absorption band with $\lambda_{max} = 250$ nm increases with time (Fig. 3A, spectra 1–5). During this time period, the time-dependence of absorption spectra shows no isosbestic points such as those that appear at pH 9.3 at ammonia concentrations higher than 0.1 M. Only after more than 120 s, where the slower reaction predominates (Fig. 3B, spectra 6–10), isosbestic points are observed. The changes in spectra in the course of the slower reaction are discussed below. This indicates that the species absorbing at 250 nm is not an intermediate in the sequence of consecutive equilibria involved in the slower reaction, but a product of a competitive, side-reaction that reaches an equilibrium. The absorption band at 250 nm is attributed to an imine, resulting in dehydration of the primarily, even more rapidly formed carbinolamine.

At pH 8.23 in a solution containing 0.001 M NH_3 and 0.01 M NH_4Cl and 3.0 M KCl, where the rate of the slower reaction (see below) is negligible, the decrease of concentration of OPA was followed by an increase in absorbance at 257 nm. This reaction follows first order kinetics§ with a rate constant $k_{obs} = 5.8 \times 10^{-3}$ s^{-1}.

The nature of the side product absorbing at 250 nm was confirmed by polarography. In this technique, an imine is most frequently reduced at the dropping mercury electrode at potentials more positive than that of the parent carbonyl compound.[5] When the reaction between OPA and NH_3 was studied in ammonia–ammonium chloride buffers at pH 9.3 at $[NH_3] < 0.1$ M, a more positive wave i_1' was observed. This wave i_1', which corresponded to the reduction of the imine, occuring at potentials about 0.15 V

‡ Rate of hydration of OPA (current i_1) was compared to the decrease in current i_1 after acidification to pH 4.7 of a product formed in the slower reaction of OPA in solution containing 1×10^{-4} M OPA (Supplementary Data A).†

§ The role of pH at $[NH_3]$ = const on the rate of the fast initial reaction of OPA with NH_3 was followed (Supplementary Data B).†

Table 1 Dependence of the first order rate constants of the fast reaction on concentration of ammonia in buffers containing 1×10^{-4} M orthophthalaldehyde at pH 9.3 (at equal concentrations of NH_3 and NH_4Cl) determined from increase in absorbance at 250 nm (k'_{250}) and from increase of current i_1'. For $k' = k_{obs}$ [NH_3], it follows that $k' = 1.1 \times 10^2$ L mol^{-1} s^{-1} ($R^2 = 0.98$)

[NH_3]	0.02	0.03	0.04	0.06	0.1
$k'_{280} \times 10^3$ s^{-1}	6.4	7.4	9.0	10.4	15.6
$k'_i \times 10^3$ s^{-1}	6.3	—	8.8	9.6	15.4

more positive than wave i_1, can be observed only in initial stages of the reaction. The half-wave potential of wave i_1 is practically the same as those obtained in solutions of OPA in borate or 4-hydroxybenzene sulfonate at the same pH 9.3, where formation of imine would not take place.

The proof of the nature of the short-lived species, that absorbs at 250 nm and is reduced in wave i_1', is supported by comparison of the kinetics of its formation followed spectrophotometrically at 250 nm with the kinetics of formation of the species reduced in polarographic wave i_1' (Fig. 4). Such comparisons were restricted to solutions containing between 0.01 M and 0.1 M NH_3, where the slower reaction does not interfere. Under such conditions, the time-dependence of the absorbance at 250 nm parallels the increase in the polarographic wave i_1', which is proportional to the concentration of the imine. Furthermore, the values of the first order rate constants, obtained from the increase in absorbance at 250 nm at each concentration of NH_3, are in good agreement with values obtained from the increase of the polarographic current i_1' with time (Table 1). The dependence of the average rate constant k_{obs} on concentration of ammonia in the buffer is linear ($R^2 = 0.985$) and corresponds to a second order rate constant $k = 1.1 \times 10^2$ L mol^{-1}s^{-1}.

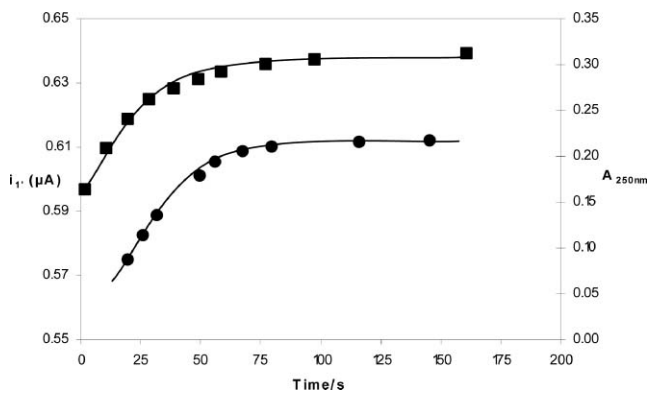
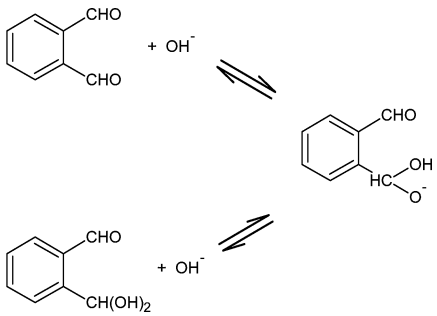


Fig. 4 Dependence of current i_1' and absorbance at 250 nm for a fast reaction of 1×10^{-4} M orthophthalaldehyde with NH_3 in a buffer pH 9.3, composed of 0.1 M NH_3 and 0.1 M NH_4Cl. The ionic strength was kept constant at 0.1 M. Comparison of the time dependence of (1) limiting polarographic currents i_1' (left hand scale) with that of (2), the absorbance of the imine at 250 nm (right hand scale).

The initial rate of the fast reaction (v_o) increases with increasing pH. This increase resembles that obtained from the pH-dependence of the rate of dehydration of OPA.[4]¶ This indicates that the rate of formation of the imine is controlled by the rate of dehydration of OPA.

The rate of the slower reaction decreases with increasing pH (see below). This enables the following of the fast reaction using an increase of absorbance at 250 nm even in the presence of 0.3 M NH_3 or 1 M NH_3 at pH 9.8. In such solutions, the formation of the imine had the rate constant $k_{obs} = 1.1 \times 10^2$ s^{-1}, the same as obtained from absorbance at 250 nm. More extensive investigation of the rate of pH in more alkaline solutions was prevented by the competitive reactions of OH^- ions with OPA eqn (2).



(2)

The slower reaction of OPA in the presence of ammonia

The time-interval during which the slower reaction between OPA and NH_3 predominates depends on concentrations of both NH_3 and NH_4Cl. The higher the concentration of NH_3 and/or the lower the pH (resulting in a smaller ratio of [NH_3] : [NH_4^+]), the shorter is the time after mixing after which the slower reaction predominates.

The slower reaction can be conveniently followed by polarography by recording current–voltage curves at chosen time intervals. On such curves, wave i_1 of the unhydrated form of OPA **1** and i_2 of the hydrated acyclic form **2** decrease with time, whereas wave i_3 of the reduction product at about −1.6 V (at pH 9.3) increases (Fig. 5).

The decreases of waves i_1 and i_2 and the increase in i_3 follow first order kinetics with the same rate constant k_{obs} (Table 2). This indicates that the equilibria between forms **1**, **2** and **3** are established rapidly relative to the reaction of the unhydrated form **1** with ammonia. Absence of wave i_1' of the reduction of imine **4** at times longer than about 5 minutes indicates conversion of the imine **4** back into the carbinolamine **7**, which undergoes cyclization into **9a** and **9**. Hence the decrease in current i_1 is proportional to the decrease in the total concentration of the OPA due to its reaction with ammonia. Increase in the current i_3 corresponds to the increase in concentration of the reducible species **9** with increasing concentration of NH_3. As the rate constant $k_{obs} = 9.2 \times 10^{-4}$ L mol^{-1} s^{-1} for the decrease in [**1**] is practically identical with a rate constant $k_{obs} = 9.0 \times 10^{-4}$ L mol^{-1} s^{-1} obtained under the same conditions for the increase in [**9**], it can be concluded that in the conversion of **1** into **9**, there is no accumulation of an intermediate, such as carbinolamine **7** or that of **9a** nor of the side product, the imine **4**.

¶ The dependence of the rate of dehydration of OPA in a 1×10^{-4} M solution on pH with the pH-dependence of the rate of the initial reaction of 1×10^{-4} M OPA with 0.02 M NH_3 in the presence of 0.02 M NH_4Cl was compared at pH 9.3 at ionic strength $\mu = 0.2$ (Supplementary Data C).†

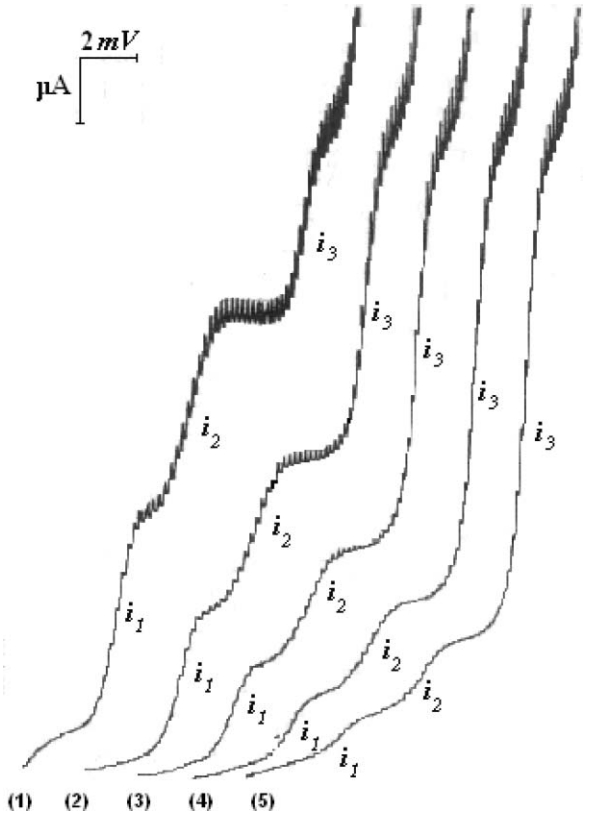


Fig. 5 Time dependence of polarographic current–voltage curves in solutions of 1×10^{-4} M orthophthalaldehyde in a solution of 0.1 M NH_3 and 0.1 M NH_4Cl. Curves starting at –0.6 V after (1) 5.5 min, (2) 16 min, (3) 23 min, (4) 34 min, (5) 42 min after mixing. $dE/dt = -0.6$ V s^{-1}. Waves i_1 and i_2 are due to the reduction of the starting dialdehyde, waves i_3 to the product—dehydrated 1,3-dihydroxyisoindole.

In the view of some uncertainty in the values of currents i_1 and i_3 under conditions when equilibria are established (resulting from the role of the very slow reactions described below), values of k_{obs} obtained from measurements of half-times were included in Table 2. Hence polarographic kinetic experiments also indicate the absence of any measurable accumulation of intermediates in the equilibria in the reactions of OPA with NH_3 yielding an isoindole derivative.

The slower reaction between OPA and NH_3 in ammonia–ammonium chloride buffers, pH 8.3 to about 10.0, was also followed by spectrophotometry. During time-intervals between about 3 min and 90 min, we observed increasing absorbances at 240 and 284 nm and decreasing absorbances at 264 and 294 nm. All these absorptions correspond to $\pi \rightarrow \pi^*$ transitions. Absorptions at 264 and 294 nm were attributed to OPA. This was confirmed by comparison of spectra of equimolar solutions of OPA in an ammonia–ammonium chloride buffer pH 9.3 and in a borate buffer pH 9.3. The absorptions at 240 and 284 nm thus correspond to the product of the reaction of OPA with NH_3, the isoindole derivative **9**.

To avoid complications due to an overlap of the band at 240 nm by the band of the imine (see above) at 250 nm during the initial stages of the reaction (Fig. 3A), the kinetics of formation of the isoindole product **9** were followed by measurement of the increase of absorbance at 284 nm. Kinetics of the conversion of OPA were

Table 2 First order rate constants (k_{obs}) for the slower reaction of 0.1 mM orthophthalaldehyde at varying concentrations of ammonia at pH 9.3 at 25 °C. Buffers contain equal concentrations of ammonia and ammonium chloride

$[NH_3]$	$[NH_4^+]$	[KCl]	$k_{obs} \times 10^4$ s^{-1}				Mean $k_{obs} \times 10^4$ s^{-1}
1.00	1.00	—	8.4[a,b]	9.6[a,c]	8.2[d,e]	9.3[d,f]	8.9
0.30	0.30	—	3.0[a,b]	3.1[a,c]	3.0[d,e]	3.1[d,f]	3.2
			4.0[g,h]	3.0[g,i]	3.0[j,k]	3.3[j,l]	
0.20	0.20	0.10	2.0[a,b]	2.1[a,c]	2.0[d,e]	2.2[d,f]	2.2
			3.0[g,h]	2.0[g,i]	2.0[j,k]	2.3[j,l]	
0.10	0.10	0.20	1.0[a,b]	1.3[a,c]	0.9[d,e]	1.2[d,f]	1.2
			2.0[g,h]	1.1[g,i]	1.0[j,k]	1.2[j,l]	
0.08	0.08	0.25	0.67[a,b]	0.96[a,c]	0.72[d,e]	0.89[d,f]	0.81
0.06	0.06	0.24	0.62[a,b]	0.69[a,c]	0.67[d,e]	0.67[d,f]	0.66
0.05	0.05	0.25	0.60[a,b]	0.49[a,c]	0.50[d,e]	0.48[d,f]	0.52
0.02	0.02	0.28	0.33[a,b]	0.28[a,c]	0.33[d,e]	0.29[d,f]	0.31

[a] Values of rate constants for the decrease in concentration of orthophthalaldehyde from variations of polarographic limiting currents i_1 with time. [b] Values of k_{obs} from $\log(i_1 - i_1^e) = f(t)$. [c] Values of k_{obs} from $i_1 = f(t)$, $r_{1/2}$. [d] Rate constants from the increase in polarographic limiting current i_3 with time. [e] Values of k_{obs} from $\log(i_3 - i_3^e) = f(t)$. [f] Values of k_{obs} from $i_3 = f(t)$, $r_{1/2}$. [g] Values of k_{obs} for the decrease in concentration of the orthophthalaldehyde from variations of absorbance at 285 nm. [h] Values of k_{obs} from $\log(A_{285} - A^e_{285}) = f(t)$. [i] Values of k_{obs} from $A_{285} = f(t)$, $r_{1/2}$. [j] Values of k_{obs} for the increase in absorbance of the reaction product at 262 nm. [k] Values of k_{obs} from $\log(A_{262} - A^e_{262}) = f(t)$. [l] Values from $A_{262} = f(t)$, $r_{1/2}$.

studied using the decrease of absorbance at 264 nm, as this band has a higher molar absorptivity than that found at 294 nm.

When the concentration of NH_3 in the buffer was higher than about 0.3 M at pH 9.3 (that is at equal concentrations of ammonia and ammonium chloride), the role of the fast reaction manifested by formation of the band at 250 nm can be neglected. Under such conditions, the decreases of the bands at 264 and 294 nm and the increases of those at 240 and 284 nm in the course of the reaction, result in formation of three isosbestic points at 250, 274 and 289 nm (Fig. 3B). Such a pattern of changes of spectra with time indicates that, under the conditions used, both the concentration of the imine and that of any intermediate in the sequence of consecutive equilibria are negligible. This confirms the deduction based on polarographic data (see above). Thus in the reaction of OPA with ammonia, the starting materials are in equilibrium with the isoindole product **9**.

This conclusion is further supported by the fact that both the decrease in concentration of OPA indicated by the decrease of the absorbance of 264 nm and that of the increase in concentration of the isoindole product based on absorbance at 284 nm follow first order kinetics. Moreover, the observed rate constants for the decrease in concentration of OPA and for the increase in concentration of the isoindole derivative were found to be equal (Table 2).

The reason for the immeasurably low concentration of the imine **4** and of the intermediates **7** and **9a** is the larger stability of the isoindole product. Its formation is the driving force of the sequence of equilibria, resulting in a simple overall equilibrium.

The rate of the establishment of the equilibrium between OPA **1** and the isoindole derivative **9** at pH 9.3 is first order in concentration of NH_3. This is shown by the linear dependence of values of k_{obs} (Table 2) on concentration of ammonia at pH 9.3, which follows the equation $k_{obs} = k_1$ $[NH_3]$. For $k_1 = k_{obs}/[NH_3]$

Table 3 Dependence of first order rate constants (k_{obs}) on pH for the slower reaction of 0.1 mM orthophthalaldehyde with ammonia at 25 °C. Buffers contained 0.1 M NH_3 and a varied [NH_4Cl] at $\mu = 1.0$

[NH_3]	[NH_4^+]	[KCl]	pH	[H^+] × 10^9	$k_{obs} \times 10^4$ s^{-1}				Mean $k_{obs} \times 10^4$ s^{-1}
0.1	1.00	—	8.3	5.0	5.7[a,b]	7.7[a,c]	5.8[d,e]	6.4[d,f]	6.4
0.1	0.79	0.2	8.4	4.0	4.5[a,b]	6.7[a,c]	4.6[d,e]	5.8[d,f]	5.4
0.1	0.30	0.7	8.8	1.6	2.3[a,b]	2.3[a,c]	2.5[d,e]	2.5[d,f]	2.3
0.1	0.10	0.9	9.3	0.50	1.0[a,b]	1.6[a,c]	1.1[d,e]	1.2[d,f]	1.2
0.1	0.03	0.97	9.8	0.16	0.43[a,b]	0.57[a,c]	0.43[d,e]	0.58[d]	0.5

[a] Values of rate constants for the decrease in concentration of orthophthalaldehyde from variations of polarographic limiting currents i_1 with time. [b] Values of k_{obs} from $\log(i_1 - i_1^e) = f(t)$. [c] Values of k_{obs} from $i_1 = f(t)$, $r_{1/2}$. [d] Rate constants from the increase in polarographic limiting current i_3 with time. [e] Values of k_{obs} from $\log(i_3 - i_3^e) = f(t)$. [f] Values of k_{obs} from $i_3 = f(t)$, $r_{1/2}$.

and $y = 1.00$, the value of $k_1 = 9.1 \times 10^{-4}$ L mol^{-1} s^{-1} has been found.

Changes of absorbance with time at about 224 nm and in the wavelength region corresponding to forbidden n → π* transitions at $\lambda > 300$ nm were not taken into consideration. At these wavelengths, absorption bands were due to overlap of several species. Thus the absorbance in the range between 300 and 320 nm is a result not only of transition involving the two formyl groups of the unhydrated OPA, but also of transition that involves the carbonyl groups in all benzaldehyde derivatives participating in the reaction, including the monohydrated acyclic form **2**.

The role of pH on the rate of the slower reaction between OPA and NH_3 was investigated in buffers, in which the concentration of NH_3 and the ionic strength were kept constant and the concentration of NH_4^+ ions was varied. The values of the rate constant k_{obs} obtained from plots of log ($i_1 - i_1^e$) and log ($i_3 - i_3'$) as a function of time were comparable (Table 3) as were the values obtained from $\tau_{1/2}$. The rate of the establishment of the equilibria, in which OPA and NH_3 yielded the isoindole derivative, increases with decreasing pH. The reaction is thus acid catalyzed. To distinguish between a specific and a general catalysis, the rate constant of the slower reaction in the ammonia–ammonium ion buffers was determined at varying concentration of ammonium ions at varying pH of these solutions. The first order rate constant (k_{obs}) in such buffers at varying ratios of [NH_3] and [NH_4^+] was a linear function of both [NH_4^+] and [H^+].

At varied pH, the value of k_{NH4+} obtained from the intercept of $k_{obs} = f([H^+])$ was found to be practically the same at various pH. It can be thus concluded that the increase in the rate of formation of **9** is due to a general acid catalyzed ring formation, **1** → **9** or **2** → **9**.

Reactions of OPA with 2-aminoethanol (RNH_2)

At pH 9.5 (at [RNH_2] = [RNH_3^+]) when excess of the concentration of RNH_2 was five times or more higher than the concentration of OPA, and the reaction followed first order kinetics, the establishment of the equilibria was too fast to be followed by available techniques. The reactions were too fast even under conditions of second order kinetics, where [OPA] = [RNH_2] at pH 9.5. Reactions such as the latter were carried out in solutions containing 0.1 mM OPA and 0.2 mM RNH_2 buffered by borate or 4-hydroxybenzene sulfonate.

The kinetics of the reaction between OPA and RNH_2 could have been followed in a 4-hydroxybenzene sulfonate buffer pH 8.5, to which equal concentrations (0.1 mM) of OPA and 2-aminoethanol were added. In such a reaction mixture, OPA is present in about 10 times excess over the reactive form of RNH_2. In such reaction mixtures, the initial reaction velocity (υ_o) was determined from the decrease of wave i_1 of OPA (which is proportional to its concentrations) with time. The value of υ_o is a linear function of concentration of the 2-aminoethanol. This observation [at a pH 8.5 which is smaller than the pK_a of the amine (9.5)] indicates that the rate determining step is the addition of an unprotonated RNH_2 to one of the carbonyl groups of the unhydrated form of OPA. The interaction of OPA with RNH_2 is thus slower than the rate of the establishment of equilibria between **1**, **2**, and **3**. The slope of the $\upsilon_o = f([RNH_2])$ plot corresponds to a $k_{obs} = 3 \times 10^1$ L mol^{-1} s^{-1} with $R^2 = 0.993$.

Spectrophotometry enables the following of the reaction of OPA with 2-aminoethanol (RNH_2), even in the absence of a higher concentration of the buffer and that of a supporting electrolyte. It enables us to avoid the addition of a borate or *p*-hydroxybenzene sulfonate buffers at pH 9.5. The general acid–base catalytic properties of such added buffers would complicate the investigated kinetics. Thus to follow the kinetics in a solution containing 0.005 M RNH_2 and 0.005 M RNH_3^+ at pH 9.5, it was possible to measure changes in absorbances at 235 and 285 nm, corresponding to absorption due to the product **9**. The increases in absorbance followed first order kinetics‖ with $k_{obs} = 5.7 \times 10^{-3}$ s^{-1}, corresponding to a second order rate constant $k = 1.1 \times 10^{\circ}$ L mol^{-1} s^{-1}.

Because the rate of decrease in [OPA] corresponds to an increase of the isoindole derivative reduced in wave i_3, no accumulation of intermediates occurs, similar to the reaction with ammonia. This reaction leading to an equilibrium is followed by an irreversible process, corresponding to the very slow reaction observed in the reaction with ammonia. The rate of this consecutive reaction increases with increasing concentration of RNH_2. Thus in solution containing 1×10^{-4} M OPA, 1×10^{-3} M RNH_2, 1×10^{-3} M RNH_3^+ and 1.0 M KCl, the reduction of the product of the reaction of OPA with RNH_2 occurs at –1.65 V. With increasing concentrations of RNH_2 and RNH_3^+ at constant pH and $\mu = 1.0$, the wave i_3 at –1.65 V (recorded after 1 h) gradually decreases (Fig. 6) and a new wave, i_3' at –1.60 V, increases. The equal limiting currents of waves i_1 (at [RNH_2] < 0.1 M) and i_1' (at [RNH_2] > 0.5 M) indicate that in both processes, the same number of electrons ($n = 2$) is transferred.

‖ Reaction of 2-aminoethanol (RNH_2) with OPA was followed in a solution containing 0.005 M RNH_2, 0.005 M RNH_3^+ (pH 9.5) and 1×10^{-4} M OPA (Supplementary Data D).†

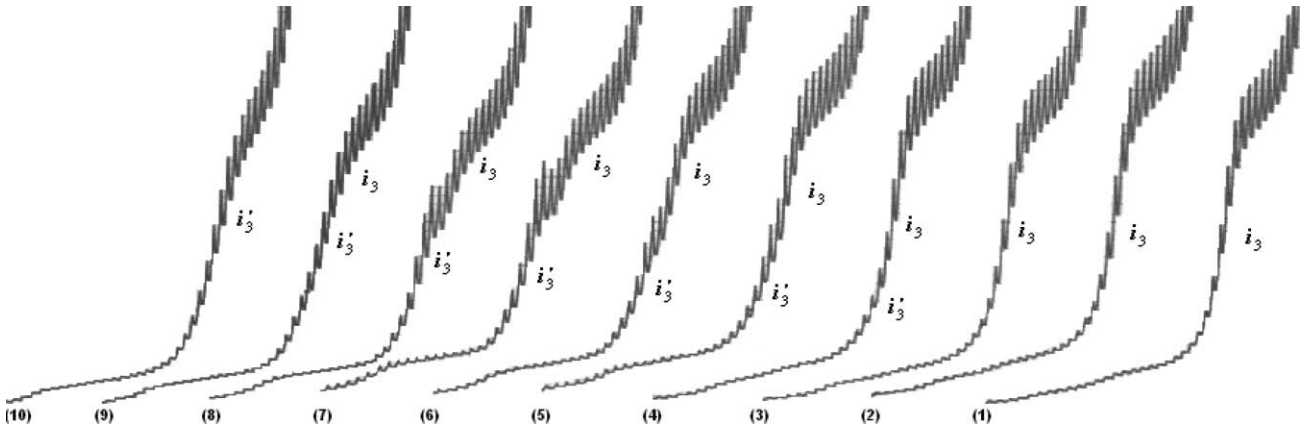


Fig. 6 Dependence of polarographic current–voltage curves in solutions of 1×10^{-4} M orthophthalaldehyde on concentration of 2-aminoethanol in solutions of (1) 0.001 M, (2) 0.002 M, (3) 0.005 M, (4) 0.01 M, (5) 0.02 M, (6) 0.05 M, (7) 0.1 M, (8) 0.2 M, (9) 0.5 M and (10) 1.0 M of the reactive form of 2-aminoethanol at equal concentrations of its protonated form. Curves starting at −1.0 V recorded 2 hours after mixing when equilibrium is established. Wave i_3' is due to reduction of product of consecutive reactions occurring at higher concentration of 2-aminoethanol while i_3 is due to reduction of the primary product, the dehydrated 1-3-dihydroxyisoindole.

The similar half-wave potentials, the similar wave-shapes as well as similar UV spectra indicate the presence of a similar conjugated system and a similar type of a reducible grouping. Due to re-establishment of equilibria between **1** and **9** and the presence of consecutive reactions, isolation and identification of species reducible in wave i_3 and i_3' and absorbing at 235 and 285 nm has not been successful so far.

Very slow reactions

In interactions between OPA and NH_3 over periods of time longer than about 2 h, very slow consecutive reactions take place. This is manifested by a slow decrease of the polarographic limiting currents i_3 with time as well as of the absorbance at 262 nm. Both these quantities are attributed to the dehydration product **9** of the 1,3-dihydroxyisoindole. These very slow reactions result in formation of species absorbing at 301, 330 and 430 nm (Table 4). In these reactions, an increase in absorbances at about 550 and 650 nm is also observed.

Time periods in which the role of consecutive reactions yielding the above mentioned species become significant, are much longer than those encountered in real life practical applications. Therefore a further preliminary investigation of very slow processes was mostly limited to those that can play a role at time periods shorter than about 3 hours.

The very slow reactions were investigated in three types of reaction mixtures, all containing 0.1 mM OPA. (a) The reaction of OPA with NH_3 was carried out in a solution containing 0.3 M ammonia and an equal concentration of ammonium chloride at pH 9.3. Under these conditions, the OPA is completely converted into dehydrated form **9** of 1,3-dihydroxyisoindole. (b) The reaction of OPA with 2-aminoethanol (RNH_2) was carried out at pH 9.5 in solutions containing 1 mM RNH_2, 1 mM RNH_3^+ and 1.0 M KCl to control the ionic strength. In such solutions, wave i_3 predominates. Its limiting current is proportional to the concentration of the dehydrated form of the N-substituted 1,3-dihydroxyisoindole **9**. (c) When the reaction was carried out also at pH 9.5 in solutions containing 0.5 M RNH_2 and 0.5 M RNH_3^+, the isomer of **9** was formed in an irreversible process, which is reduced in wave i_3'.

Table 4 Time dependence of limiting currents (i_3), half-wave potentials of wave i_3 $(E_{1/2})_3$, absorbances at several wavelengths and of florescence at two wavelengths for the slow reaction of 1×10^{-4} M OPA on concentrations of ammonia and 2-aminoethanol (RNH_2)

0.3 M NH_3, 0.3 M NH_4Cl

t/h	$i_3/\mu A \times 10^1$	$i_3 E_{1/2}$/V	$A_{301nm} \times 10^2$	$A_{330nm} \times 10^2$	$A_{430nm} \times 10^2$	$^aF_{315nm} \times 10^2$	$^bF_{455nm}$
0.5	—	−1.6	—	—	—	1.19	2.13
0.6	3.65	—	3.11	1.17	1.80	—	—
1.0	6.30	—	—	—	—	1.02	1.95
1.3	6.81	−1.59	2.86	1.03	1.81	—	—
1.5	—	−1.58	—	—	—	—	—
2.0	—	−1.57	—	—	—	1.22	2.05
2.3	7.39		2.89	1.12	1.82	—	—
3.0	—	—	2.93	1.15	1.85	1.01	2.03
4.5	8.13	−1.57	2.94	1.21	1.79	—	—
24.0	—	—	2.75	1.16	1.23	—	—
48.0	—	—	2.70	1.21	1.11	—	—
96.0	—	—	2.93	1.47	1.16	—	—

[a] Intensity of florescence at 315 nm, excitation at 230 nm. [b] Intensity of absorbance at 455 nm, excitation at 340 nm.

(a) Under these conditions, the half-wave potential of wave i_3 is gradually shifted with time to somewhat more negative potentials. Simultaneously, the absorption band at 262 nm attributed to the 1,3-dihydroxy derivative slowly decreases (Table 4). The initial decrease of the band at 330 nm is followed by a consecutive increase at times longer than 2 h. Simultaneously, the band at 430 nm that remains unchanged over 3 h, decreases at longer times.

(b) The half-wave potential of wave i_3 shows a small shift to more positive values with time (Table 4). This process is accompanied by a decrease of the band of the dehydrated 1,3-dihydroxyisoindole at 262 nm. Products of this reaction absorb at 301 and 330 nm; these absorption bands increase with time. Under these conditions the intensity of fluorescence at 315 nm (for excitations at 230 nm) and at 455 nm (excitation at 340 nm) did not show gradual changes. (c) The half-wave potential of wave i_3' is shifted gradually with time to more positive potentials (Table 4). This shift is accompanied by the increase in absorbance at 262 nm. Under these conditions, the absorption bands at 301 and 330 nm decrease (Table 4). This behaviour confirms that the isomer reduced in wave i_3' differs from that reduced in wave i_3.

All the observations above stress the importance of control of the time between the preparation of the reaction mixture and the measurement in the development of analytical methods for determination of amines using OPA as a reagent.

Experimental

Chemicals

Orthophthalaldehyde (OPA) was purchased from Ralph N. Emanuel LTD Research Chemicals. Acetonitrile was supplied by J. T. Baker; it was used to prepare 0.01 M stock solutions for OPA.

Boric acid, sodium and ammonium chloride, ammonia, sodium hydroxide and sodium acetate, for the preparation of buffers were reagent-grade chemicals obtained from J. T. Baker; glacial acetic acid was obtained from VWR Scientific Inc.; 2-aminoethanol hydrochloride and 4-hydroxybenzenesulfonic acid were purchased from Aldrich. The sample of isoindolin-1-one was kindly supplied by J. Urban (J. Heyrovsky Institute of Physical Chemistry, Czech Academy of Science, Prague, Czech Republic).

Instrumentation

For recordings of DC current–voltage curves, a Sargent-Welch Model 4001 Polarograph was used. For recording of the current–voltage curves, the investigated solution was placed, together with the capillary of the dropping mercury electrode, into the working compartment of the Kalousek cell. In this cell, the working compartment is connected by a liquid junction with the compartment containing the reference electrode. A saturated calomel electrode (SCE) was used as the reference electrode. The dropping mercury electrode (DME) had a drop-time of $t_1 = 4$ s and $m = 2.2$ mg s^{-1} at $h = 49$ cm.

UV–vis absorption spectra were recorded using a Hewlett-Packard Agilent 8453 UV–vis spectrophotometer. Fused quartz cells were used with a 10 mm path-length.

For measurement of fluorescence, a Perkin Elmer Luminescence Spectrometer LS 50B was used. The excitation wavelength for the fluorescent species was chosen to be 230 nm for ammonia buffer, producing a species fluorescent at 350 nm. For a system buffered by 2-aminoethanol, the excitation at 330 nm resulted in fluorescence at 435 nm. A Denver Instrument, model UB-10, pH meter equipped with a glass electrode, was used for the pH measurements.

Solutions

Stock solutions (0.01 M) of OPA were prepared in acetonitrile. All stock solutions were stored in a refrigerator in the dark and used in less than two weeks.

Ammonia and 2-aminoethanol buffers of pH 9.3 and pH 9.5, respectively, were prepared by using the same ratios of the amine and the ammonium form, but varying the concentration of the amine. At low concentrations of the ammonium form, the pH was kept constant by addition of a borate or 4-hydroxybenzenesulfonate buffer of the same pH and ionic strength constant by addition of potassium chloride solution.

For investigation of the role of pH, the concentration of the amine was kept constant and the concentration of the protonated form of the amine was varied.

General procedures

For recordings of polarographic *i–E* curves, 0.1 mL of a 0.01 M stock solution of the OPA were added to 9.90 mL of the supporting electrolyte. In most instances, a solution of ammonia or the amine containing the corresponding conjugate acid were used both as a reagent, a buffer and a supporting electrolyte. Only at amine concentrations lower than 0.005 M borate or a 4-hydroxybenzene sulfonate was added. Potassium chloride was added to keep the ionic strength at $\mu = 0.1$ or 1.0.

For the determination of the equilibrium constants, the dependence on the concentration of NH_3 or RNH_2 was studied. The solutions were left to reach equilibrium for one day in ammonium buffer and two hours in 2-aminoethanol buffer. After purging with nitrogen gas for about 3 min to remove the oxygen, the polarographic current–voltage curves were recorded.

For recording of absorption spectra in the UV region, 0.1 mL of a 0.01 M stock solution were added to 9.90 mL of ammonia and 2-aminoethanol buffers at pH 9.3. The solutions were allowed to reach equilibrium for one day and 2 hours in ammonia and 2-aminoethanol respectively.

Procedures for kinetic studies

For the kinetic studies of OPA with ammonia and 2-aminoethanol, rate constants were determined for three different stages: the *fast*, *slower* and *very slow* reactions.

Fast reactions. The kinetics of the decrease of the starting material of 1×10^{-4} M OPA in ammonia buffers of pH 9.3 with [NH_3] varied between 0.1 and 0.02 M were followed within the first 120 s using polarography at constant potential (–0.99 V). The current was recorded as a function of time. At concentrations of NH_3 higher than 0.1 M, the reactions were too fast to be followed. UV–vis spectroscopy also enabled us to follow the kinetics of the fast reaction of 1×10^{-4} M OPA in ammonia buffers of pH 9.3 with [NH_3] between 0.1 and 0.02 M. Measurements of the absorbance were taken every 7 seconds.

The kinetics of the decrease of the starting material of 1×10^{-4} M OPA in the presence of 2-aminoethanol were too fast to follow, even under conditions for a second order reaction at concentration of the 2-aminoethanol equal to 1×10^{-4} M. To reach a measurable rate, solutions of 1×10^{-4} M OPA and 1×10^{-4} M 2-aminoethanol were prepared in a phosphate buffer pH 8.5. Under such conditions, the concentration of OPA was tenfold higher than that of the reactive, unprotonated form of 2-aminoethanol ($pK_a = 9.5$).

Slower reactions. The kinetics of the slower reactions were followed between 3 min and 120 min using both polarography and UV–vis spectroscopy. Using ammonia buffers at pH 9.3, the concentration of ammonia was varied between 0.5 M and 0.01 M. Polarography allowed us to follow the decrease of the starting materials by following waves i_1 and i_2 and the increase of the product formed at −1.6 V (i_3).

UV–vis spectroscopy was also used to follow reactions of 1×10^{-4} M OPA in ammonia buffers of pH 9.3 for concentrations of ammonia between 0.5 M and 0.01 M. The absorbances at 235, 250, 262, 282 and 301 nm were recorded.

For following the dependence of the reaction of 1×10^{-4} M OPA with NH_3 on the pH, ammonia buffers between pH 8.3 and 10.3 were prepared, keeping the [NH_3] constant at 0.3 M and keeping the ionic strength constant at $\mu = 0.95$.

Very slow reactions. The very slow reactions were followed for periods of between hours and days for both 1×10^{-4} M OPA in ammonia and 2-aminoethanol buffers of pH 9.3 and 9.5 respectively.

Fluorescence measurements

The fluorescence was followed in solutions containing 1×10^{-4} M OPA and amine–ammonium ion buffers, one hour after mixing. Under these conditions, the equilibria between OPA and the amine were established. In particular, the fluorescence was followed of products that were formed in 0.3 M NH_3 and 0.3 M NH_4Cl (which are reduced at −1.65 V). Alternatively, the fluorescence was measured of species formed in solutions containing 0.001 M RNH_2 and 0.001 M RNH_3^+ (which are reduced at −1.65 V) as well as those generated in solutions of 0.5 M RNH_2 and 0.5 M RNH_3^+ (which are reduced at −1.60 V).

Reversibility test

For determination of reversibility, the reaction of 0.1 mL of 0.01 M OPA in 9.90 mL of ammonia pH 9.3 or 2-aminoethanol buffer at pH 9.5 was allowed to reach equilibrium. After it was established, 10 mL of acetate buffer pH 4.8 was added to the reaction mixture, resulting in a pH of about 4.8, and polarographic curves and UV–vis spectra were recorded. The polarographic curves and the UV–vis spectra were compared to the polarographic curves and spectra obtained for 0.1 mL of 0.01 M OPA in 10 mL of an acetate buffer pH 4.8 in the absence of the amine.

Conclusion

This investigation demonstrated the power of the combination of spectrophotometry with polarography in following equilibria and kinetics of complex reactions of organic compounds in protic solvents. The reactions involved proved to be both more complex and different from these proposed[2] to take place for the analytically important reactions of amines with orthophthalaldehyde. As opposed to the mechanism proposed[9] for the reaction of ninhydrine with amines, as well of the assumption that imine formation plays an important role in the cyclization yielding to formation of an isoindole derivative,[10] the essential role of carbinolamine rather than that of the imine, as intermediate, has been pointed out. Previously proposed reaction schemes for the reaction of OPA with amines were based solely on product identification. In this study, following the equilibria and kinetics contributed to a better understanding of some reaction steps involved. This investigation pointed out the importance of the control of the time period elapsed between preparation of the reaction mixture and measurement of UV or fluorescence spectra widely used, for example, in determinations of other primary amines and amino acids.

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