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New Reagents for the Colorimetric Determination of Microgram Quantities of Nitrite

By

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With 4 Figures

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Established methods for the determination of nitrite ion are based on three main types of reaction:

1. Formation of a colour by reaction of the nitrite with phenolic compounds[1,2].
2. Reaction with miscellaneous reagents such as 2-amino-4-chloromethyl thiazolyl hydrochloride[3], 3-methyl-1-phenyl pyrazolone[4], and N,N,N′,N′-tetramethyl benzidine[5].
3. Formation of a diazonium compound which is then coupled with a suitable second compound.

The above methods have several disadvantages: In the first type of reaction the intensity of the colour is dependent upon the pH of the solution and does not obey Beer's Law with respect to nitrite ion concentration.

Whilst reactions of the second type give relatively stable colours whose intensities are proportional to nitrite concentration, the reagents used are generally difficult to prepare in a pure state.

Probably the best known and most widely used of the coupling reactions is the Griess method[6] using α-naphthylamine and sulphanilic acid. This has been modified by various workers including *Zeller*[7] who developed it for nitrite concentrations of not greater than 0.02 p. p. m. in waters, by repeated extraction of the colour with *n*-butanol. *Ato* and *Aoki*[8] replaced the sulphanilic acid by phenol disulphonic acid and claimed thereby to be able to determine 0.05 to 0.2 p. p. m. of nitrogen as nitrite by measurement of the very intense colour produced. In the presence of many metal ions, however, the Beer's Law relationship did not obtain; slight traces of Fe(III) and more than 50 p. p. m. of Al(III), Cu(II) and Pb(II) interfered with the formation of the colour.

Among other diazonium compounds and coupling reagents which have been suggested are the diazonium compound formed from procaine hydrochloride coupled with ethanolic thymol[9], and the diazonium compound of *N*-(1-naphthyl)-ethylene diamine coupled with sulphanilamide[10]. This latter reaction is claimed to be superior to all others involving coupling, since the colour is formed fairly quickly and is stable for approximately two days.

In all methods involving the formation of a dyestuff by coupling, the diazonium compound itself is coloured but is too unstable to be of direct use. Since diazonium compounds are thermally unstable, the methods involve cooling the reaction mixture to below 10° C so that losses are reduced to a minimum. The quantitative formation of the diazonium compound is thus slow, and the whole method is time consuming.

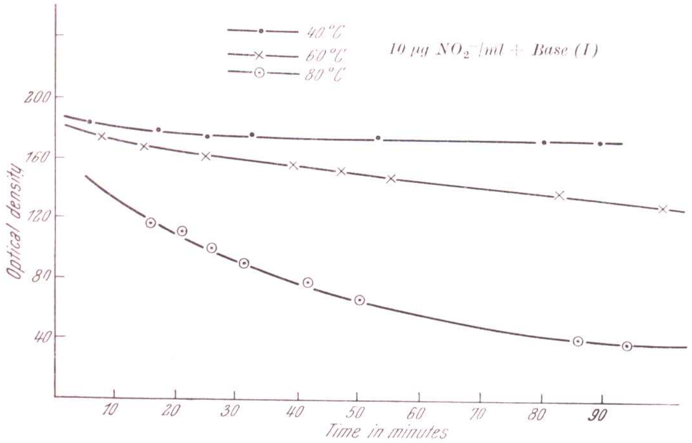

Fig. 1

The authors have investigated the use of some nitrogen heterocycles as reagents for the determination of nitrite[11]. *N*-(2-amino-phenyl) morpholine (I) and *N*-(2-amino-phenyl) piperidine (II) have proved of use in the determination of microgram quantities of nitrite ion.

Having noted that aqueous solutions of the hydrochlorides of (I) and (II) developed intense orange colours in the presence of nitrous acid, and that these colours were apparently stable for some time, the u. v. spectra of the diazonium compounds formed by (I) and (II) were examined.

Pronounced absorption peaks were noted at a wavelength of 435 nm for (I) and 445 nm for (II). The molar extinction coefficients of both compounds were approximately equal.

The rate of formation of maximum colour at various temperatures was investigated using solutions containing 10 μg NO_2^-/ml. The results are shown in Fig. 1 for (I) and Fig. 2 for (II).

From these graphs, it is apparent that for practical purposes, at room temperature, the maximum intensity of colour for (I) is developed immediately, whereas for (II) the colour develops more slowly, requiring 40 minutes to attain maximum intensity at 18° C but only 25 minutes at 30° C.

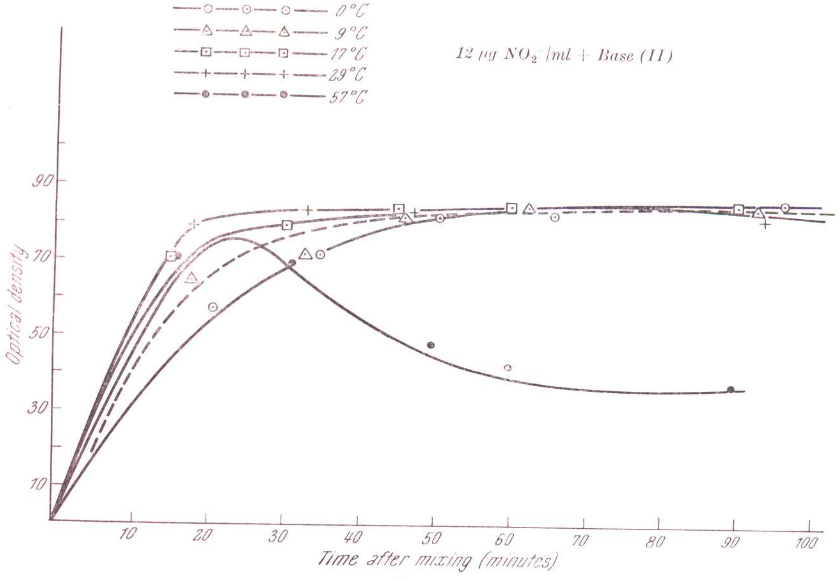

Fig. 2

Although the rate of loss of intensity, resulting from thermal decomposition of the diazonium compound, increases with temperature and becomes appreciable at higher temperatures, the relative stability of (I) is such that even at 40° C the loss in intensity after two hours is less than 7%. If the diazonium compounds are kept in the dark, the loss of intensity at room temperature is less than 1% after 24 hours.

These measurements were obtained using a thermostated cell carrier in the light-proof compartment of a spectrophotometer. However, on exposure to daylight, the intensity of the colour decreased. Results obtained from investigations of the loss of intensity on exposure to light are shown in Fig. 3 and Fig. 4. It may be seen that exposure to light for a short time (say 10 minutes) causes no appreciable loss of intensity for the colour developed with 10 μg NO_2^-/ml.

The effect of nitrite concentration on the colour was investigated using solutions of sodium nitrite containing between 0.2 and 20 μg NO_2^-/ml, mixed at room temperature with an acidic solution of (I) or (II) containing 50 μg of base/ml. Using (I), the absorption of light at 435 nm was measured immediately after adding the acid solution of the base to the nitrite. Using (II), the absorption at 445 nm was measured after the solution had been allowed to stand in the dark for 40 minutes.

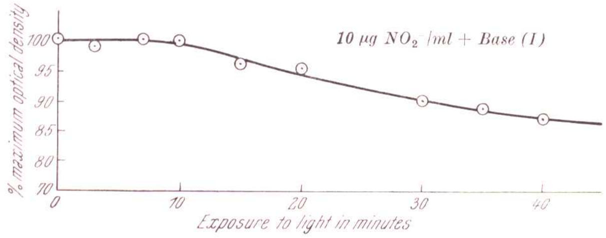

Fig. 3

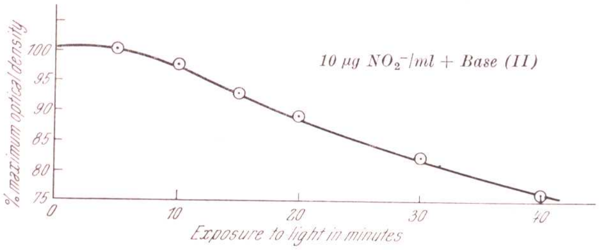

Fig. 4

In both cases the intensity of the colour produced was strictly proportional to the concentration of the nitrite used. The results were reproducible to 0.05 μg/ml, the reaction is sensitive to 0.05 μg/ml using a 1-cm light path.

The effect of substances additional to the nitrite ion was investigated and it was found that:

1. up to a 200 fold excess of either base did not interfere;

2. excess of acid did not interfere;

3. when present in concentrations of up to 200 μg/ml, the following ions had no effect on colour formation:

NH_4^+, Li^+, Na^+, K^+, *Tl^+;
Ba^{2+}, Be^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, *Pb^{2+}, Mg^{2+}, Mn^{2+};
Ni^{2+}, Sn^{2+}, Sr^{2+}, UO_2^{2+}, Zn^{2+};
Al^{3+}, Cr^{3+}, NbO^{3+}, TaO^{3+};
Ti^{4+};
F^-, Cl^-, Br^-, I^-, NO_3^-, BO_2^-;
SO_3^{2-}, SO_4^{2-}, SeO_3^{2-}, SeO_4^{2-}, TeO_4^{2-}, *WO_4^{2-}, UO_4^{2-};
PO_4^{3-}.

4. Oxidising agents generally caused interference:

100 μg/ml of MnO_4^-, VO^{2+}, H_2O_2 interfered on standing at room temperature.

25 μg/ml of Fe(III), Ce(IV), $Cr_2O_7^{2-}$ interfered immediately. Interference by Fe(III) may be prevented by addition of excess F^- before addition of the base solution.

The oxidising agents all cause a red colour whose intensity increases on warming or standing. The compound formed gives a wide absorption peak at 510 nm, and attempts to identify it by extraction into *n*-butanol and chromatographic separation on Al_2O_3 were unsuccessful[12].

Acknowledgments

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Summary

Two new reagents, *N*-(2-amino-phenyl) morpholine (I) and *N*-(2-amino-phenyl) piperidine (II), are used for determination of nitrite ion in acid solutions containing up to 20 μg NO_2^-/ml. The diazonium compound of (I) is sufficiently stable to enable measurement of absorption of light at 435 nm immediately after formation at room temperature or after standing in the dark.

The reaction is sensitive to 0.05 μg NO_2^-/ml using a 1-cm light path.

Zusammenfassung

Zwei neue Reagenzien, N-(2-Aminophenyl)-morpholin (I) und N-(2-Aminophenyl)-piperidin (II) wurden für die Bestimmung des Nitritgehaltes saurer Lösungen unter 20 μg NO_2^-/ml herangezogen. Die Diazoniumverbindung von I ist hinreichend stabil zur Bestimmung der Absorption bei 435 nm unmittelbar nach ihrer Herstellung bei Zimmertemperatur oder nach Stehen im Dunkeln. Die Empfindlichkeit entspricht 0,05 μg NO_2^-/ml bei 1 cm Schichtdicke.

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* These ions, with hydrochloric acid, gave precipitates, and these were removed before the colour was measured.

Résumé

On utilise deux nouveaux réactifs, la N-(amino-2 phényl) morpholine (I) et la N-(amino-2 phényl) pipéridine (II), pour doser l'ion nitrite en solutions acides contenant jusqu'à 20 μg NO_2^-/ml. Le composé diazonium de (I) est suffisamment stable pour permettre la mesure de l'absorption de la lumière à 435 nm, immédiatement après formation à la température ambiante ou après séjour à l'obscurité.

La réaction est sensible à 0,05 μg NO_2^-/ml en utilisant un chemin optique de 1 cm.

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