DIAMINOBENZIDINE AS A REAGENT FOR VANADIUM AND SELENIUM

by

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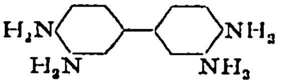
I. INTRODUCTION

The use of benzidine as a qualitative drop reagent has already been described in detail in the literature. Actually this compound, on oxidation, gives rise to a blue coloured compound having a meroquinonoid structure. Thus in acid media, one obtains a fairly selective reaction with V^{+5}-ions[1], while in alkaline media this compound can be used for detecting cerium, cobalt, manganese and silver, which also give rise to benzidine-blue by autoxidation[2].

It appeared to us that the study of other amino-diphenyl derivatives was not without importance. Hence we have studied first of all 3.4–3'.4'-tetra-aminodiphenyl, which one would expect to show a certain analogy with benzidine.



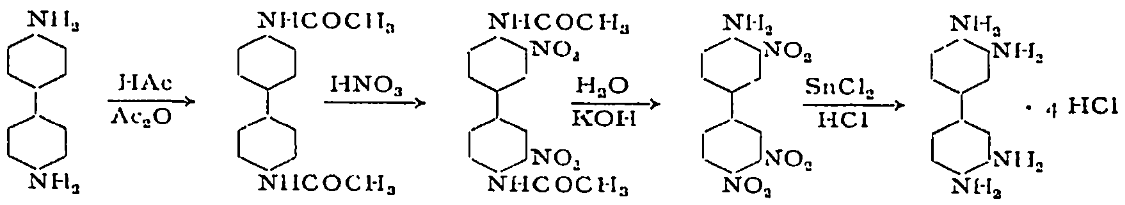
Diaminobenzidine

This compound, called diaminobenzidine by PIEN, has already been studied by him in connection with the estimation of diacetyl in butter[3, 4].

II. PREPARATION OF THE REAGENT

Since this compound is not yet available commercially, we give below a description of the preparation of diaminobenzidine as given by the previous author at the International Congress for pure and applied Chemistry held in London in 1947. When prepared in this way the compound is quite colourless and may be kept for a fair period.

A. REACTION SCHEME



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B. METHOD

1. *Acetylation of benzidine*

Benzidine, 25 g, is ground into a paste with 50 ml of glacial acetic acid and then treated slowly with 30 g of acetic anhydride, the mass becoming very warm. The resultant acetyl derivative is filtered off, boiled with alcohol to purify it and, after cooling, is filtered off and dried.

2. *Nitration*

The acetylated benzidine (30–35 g) is introduced in portions of one to two grams into 120–140 g of fuming nitric acid (d = 1.48), while the reaction mixture is cooled (temperature below 40°). The reaction mixture must be worked up quickly and as soon as the last portion is added, the solution is poured into 2–3 litres of cold water, washed and finally dried.

3. *Hydrolysis of the acetyl derivative*

The nitro-derivative (48 g) is suspended in 300 ml of alcohol and to the mixture is added 20 g of potassium hydroxide dissolved in 100 ml of water. The mixture is boiled for 5 min and on cooling, crystals separate from the red solution and are filtered off and subsequently dried.

4. *Reduction*

For 5 g of nitro-derivative a solution of 27.5 g of stannous chloride dissolved in 25 ml of 10 N-hydrochloric acid is prepared. This is heated on the water bath and the nitro-derivative is added in small quantities at a time. The reduction is complete after warming on the water-bath for a further two hours.

5. *Separation of the hydrochloride*

10 N-Hydrochloric acid, 50 ml, is added to the reduced solution, which is then allowed to stand aside and the crystalline precipitate is filtered off, redissolved in 200 ml of water, a current of hydrogen sulphide passed through the solution to remove tin, which is filtered off. The motherliquids are then treated with hydrochloric acid until at least 3 N. The chlorhydrate precipitates completely after ½ hour. It is dried in vacuum and kept in the dark. In this way the resultant product may be kept for long periods.

III DIAMINOBENZIDINE AS A REAGENT FOR VANADIUM

A. PRELIMINARY INVESTIGATION

Like benzidine, diaminobenzidine gives rise to a coloured compound on oxidation. The resultant dyestuff is violet and considerably more soluble than benzidine-blue, which makes it less suitable for a spot test on paper, since the dyestuff diffuses in the paper and the reaction thereby loses in sensitivity.

The ions mentioned in Table I were examined systematically with a 0.5% solution of diaminobenzidine hydrochloride in water (in which the hydrochloride is readily soluble) as regards their behaviour towards this reagent. Columns 1, 2 and 3 give the results in neutral, alkaline (3 N–NH_3) and acid (3 N–HCl) media, respectively. Phenomena which are to be ascribed exclusively to the ammoniacal or hydrochloric acid medium itself, were not recorded, for simplicity, unless they had a disturbing effect.

The procedure was as follows:

One drop of the solution to be examined was placed on a spot plate followed by one drop of reagent, one drop of 6 N–NH_3 or of 6 N–HCl being added before the addition of the reagent when an alkaline or acid medium was being investigated.

TABLE I

No	Ion	Compound	Conc.	1	2	3	4
1	Ag	$AgNO_3$	10^{-2}	Colourless	Brown	Colourless	Colourless
2	Hg^{I}	$Hg_2(NO_3)_2$	10^{-2}	Colourless	Brown	Colourless	Colourless
3	Cu^{II}	$Cu(NO_3)_2.2\,H_2O$	10^{-2}	Blue	Black	Blue	Blue
4	Pb	$Pb(NO_3)_2$	10^{-2}	Colourless	Colourless	Colourless	Colourless
5	Bi	$BiONO_3.4\,H_2O$	10^{-2}	,,	,,	,,	,,
6	Cd	$Cd(NO_3)_2.4\,H_2O$	10^{-3}	,,	,,	,,	,,
7	As	As_2O_3	10^{-3}	,,	,,	,,	,,
8	Sb^{III}	$SbCl_3$	10^{-2}	,,	,,	,,	,,
9	Sn^{II}	$SnCl_2.2\,H_2O$	10^{-2}	,,	,,	,,	,,
10	Au	$AuCl_3$	10^{-2}	Black	Black	Violet	Violet
11	Ru	$RuCl_3$	10^{-3}	Brown	Black	Brown	Brown
12	Rh	$RhCl_3.4\,H_2O$	10^{-3}	Deep brown	Green	Deep brown	Deep brown
13	Pd	$PdCl_2$	10^{-2}	Yellow	Black	Yellow	Yellow
14	Os	OsO_4	10^{-3}	Colourless	Colourless	Colourless	Colourless
15	Ir	$IrCl_3$	10^{-2}	Green	Green	Green	Green
16	Pt	$H_2PtCl_6.6\,H_2O$	10^{-2}	Red-brown	Black	Yellow	Yellow
17	Se	H_2SeO_3	10^{-2}	Yellow ↓	Yellow ↓	Yellow ↓	Yellow ↓
18	Te	Na_2TeO_3	10^{-2}	White ↓	White ↓	Colourless	Colourless
19	Ge	GeO_2(2 N-KOH)	10^{-3}	Colourless	Colourless	,,	,,
20	Mo	$(NH_4)_6Mo_7O_{24}.4\,H_2O$	10^{-2}	Grey	Grey-blue	,,	,,
21	W	$Na_2WO_4.2\,H_2O$	10^{-2}	White ↓	White ↓	,,	,,
22	V	NH_4VO_3	10^{-2}	Deep grey	Deep grey	Violet	*Violet*
23	Nb	Nb_2O_5(2 N-KOH)	10^{-2}	,,	Colourless	,,	,,
24	Ta	Ta_2O_5(2 N-KOH)	10^{-3}	—	,,	—	—
25	Al	$Al(NO_3)_3.9\,H_2O$	10^{-2}	Colourless	,,	Colourless	Colourless
26	Fe	$Fe(NO_3)_3.9\,H_2O$	10^{-2}	Red-brown	Black	Violet	,,
27	Cr	$Cr(NO)\,9\,HO$	10^{-3}	Violet	Grey-green	Violet	*Light violet*
28	UO_2	$UO_2(NO_3)_2.6\,H_2O$	10^{-3}	Yellow	Yellow	Yellow	Yellow
29							
30							
31	Nd	$Nd(NO_3)_3.6\,H_2O$	10^{-3}	Deep pink	Deep pink	Deep pink	Deep pink
32	La	$La(NO_3)_3.6\,H_2O$	10^{-3}	Colourless	Colourless	Colourless	Colourless
33	Ce	$Ce(NO_3)_3.6\,H_2O$	10^{-3}	,,	,,	,,	,,
34							
35	Y	$Y(NO_3)_3$	10^{-3}	,,	,,	,,	,,

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No	Ion	Compound	Conc.	1	2	3	4
36	Ti	$TiCl_4$	10^{-3}	Yellow	Yellow	Yellow	Yellow
37	Zr	$Zr(NO_3)_4$	10^{-2}	Colourless	Colourless	Colourless	Colourless
38							
39	Th	$Th(NO_3)_4 \cdot 4H_2O$	10^{-3}	,,	,,	,,	,,
40	Be	BeO	10^{-2}	,,	,,	,,	,,
41	Tl	$TlNO_3$	10^{-3}	,,	,,	,,	,,
42							
43	Ga	Ga	10^{-3}	,,	,,	,,	,,
44	In	In	10^{-3}	,,	,,	,,	,,
45	Zn	$Zn(NO_3)_2$	10^{-3}	,,	,,	,,	,,
46	Mn	$Mn(NO_3)_2 \cdot 4H_2O$	10^{-3}	,,	,,	,,	,,
47							
48	Co	$Co(NO_3)_2\,6H_2O$	10^{-2}	Pink	Pink	Pink	Pink
49	Ni	$Ni(No_3)_2 \cdot 6H_2O$	10^{-3}	Green	Green	Green	Green
50							
51	Ba	$Ba(NO_3)_2$	10^{-3}	Colourless	Colourless	Colourless	Colourless
52	Sr	$Sr(NO_3)_2$	10^{-2}	,,	,,	,,	,,
53	Ca	$CaCO_3$ (in HCl)	10^{-2}	,,	,,	,,	,,
54	Mg	$Mg(NO_3)_2\,6H_2O$	10^{-2}	,,	,,	,,	,,
55	Li	$LiCl$	10^{-3}	,,	,,	,,	,,
56	Na	$NaCl$	10^{-2}	,,	,,	,,	,,
57	K	KNO_3	10^{-3}	,,	,,	,,	,,
58	Rb	$RbCl$	10^{-3}	,,	,,	,,	,,
59	Cs	$CsCl$	10^{-3}	,,	,,	,,	,,
60	NH_4	NH_4NO_3	10^{-2}	,,	,,	,,	,,

Remarks:

1. The ions are numbered in the same order as used in the 2nd Report[1].
2. The test solutions were prepared according to the method given by NOYES[4] or by NOYES AND BRAY[5], unless otherwise stated.

From the results given in Table I it appears that diaminobenzidine in an acid medium gives a violet oxidation product with V^{+5}, Au^{+3} and Fe^{+3}. Of course, strongly oxidizing ions like Ce^{+4}, MnO_4^{-1} and CrO_4^{-2}, also react. If use is made of phosphoric acid instead of hydrochloric acid, the reaction with Fe^{+3} is eliminated; these results are collected together in Column 4.

B. DESCRIPTION OF THE REACTION

1. *Mechanism*

Diaminobenzidine gives rise to a violet coloured oxidation product, probably having a structure similar to that of benzidine-blue.

2. *Method*

On a spot plate: One drop of phosphoric acid ($d = 1.80$) and one drop of reagent are added successively to a drop of the tested solution. In the presence of V^{+5} an intense violet coloration is produced.

3. *Sensitivity and specificity*

Dilution limit: D $1 : 2.10^{-5,3}$.

In the absence of oxidising ions the reaction is specific to V^{+5}. Oxidizing ions as Au^{+3}, Ce^{+4}, MnO_4^{-1} and CrO_4^{-2}, should be avoided. Besides, the actual colour of Cr^{+3} interferes if the concentration of this element is greater than $1 : 2.10^3$. The presence of SO_4^{-2} should also be avoided since diaminobenzidine sulphate is only slightly soluble A yellow precipitate is formed with SeO_3^{-2}, so that in presence of a concentration of 10^{-2} of Se, V^{+5} can be detected with certainty only to $1 : 2.10^4$. The elements Fe^{+3}, Mo^{+6}, W^{+6}, Ti^{+4} and Mn^{+2} in a ratio of 1000 : 1 do not affect the reaction. The remaining elements in a ratio of 100 : 1 do not interfere with the reaction.

4. *Reagents*

a. 0.5% diaminobenzidine hydrochloride in water (the solid reagent can be kept indefinitely, but the solution slowly becomes coloured when exposed to air).

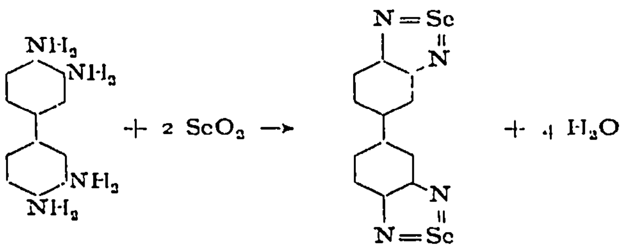
b. phosphoric acid (d = 1.80).

IV. DIAMINOBENZIDINE AS A REAGENT FOR SELENIUM

From the examination of diaminobenzidine as a reagent for V^{+5}, it has already appeared that selenous acid in either neutral, alkaline or acid media, gives rise to a yellow coloured, insoluble compound. Thanks to its great sensitivity, this reaction is of practical significance for qualitative analysis. In the EMICH tube it is indeed possible to detect Se^{+4} at concentrations down to D = $1 : 10^6$.

A. MECHANISM OF THE REACTION

Unlike most of the reactions used for the detection of selenium, the reaction described here does not depend on an oxidation-reduction process. The diaminobenzidine is not oxidised by selenous acid, but a dipiazselenole is formed, as has already been described for other ortho-diamines. With toluylene diamine, for instance, methylpiazselenole is formed, while 1.8-naphthylene diamine gives rise to the corresponding naphthylenepiazselenole[6]. When one molecule of diaminobenzidine is allowed to react in aqueous solution with two molecules of selenous acid, a yellow precipitate of diphenyldipiazselenole is formed immediately:



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1. *Identification of the piazselenole*

Diaminobenzidine hydrochloride (100 mg) is dissolved in 100 ml water and to the solution is added 44 mg of selenium (as selenous acid), a yellow precipitate of diphenyldipiazselenole being produced immediately. After standing for a little while, it is filtered, washed with water and then recrystallised twice from glacial acetic acid. The pure compound melts at 292° (uncorr.).

The dipiazselenole is insoluble in water, 6 N-HNO_3, 11 N-HCl or 6 N-NH_3 and slightly soluble in alcohol and boiling glacial acetic acid. It crystallises from the latter, on cooling, in the form of small needles, sometimes aggregated to star-like clusters. The compound is fairly stable to oxidation, but it is reduced, however, by stannous chloride giving elemental selenium. The basic properties of the compound are very weak. With 35 N-sulphuric acid, however, a red coloured salt is obtained, which immediately hydrolysed on addition of water.

2. *Analysis*

Nitrogen determination (micro-Dumas)

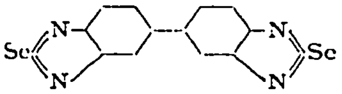
3.041 mg 0.415 ml N_2 (p 443 mm; t 22°) 15.43% N

Selenium determination

After destruction with fuming nitric acid at 250°, the solution is diluted with water, neutralised with ammonia and reduced to elemental selenium with an excess of hydrazine hydrate.

0.1170 g of product gave 0.050 g Se *i.e.*, 43.53% Se.

Calculated for



($C_{12}H_6N_4Se_2$): 15.38% N and 43.41% Se.

B. DESCRIPTION OF THE ANALYTICAL REACTION

1. *Method*

Ten drops of the solution to be examined (3 N-HCl) are introduced into an EMICH tube and three drops of the reagent are then added. In the presence of Se (as selenous acid) there is produced immediately a yellow, amorphous precipitate, or, for very low concentration (1 : 10^5 and lower), a yellow coloration. For detecting small concentrations of selenium it is essential to use a perfectly colourless reagent and it is necessary to carry out a blank. The colour intensity is a maximum only after a duration of about 5 minutes.

2. *Sensitivity and specificity*

Dilution limit: $D = 1 : 10^6 = 10^{-6}$.

The reaction is specific for Se^{+4}.

Of all the ions investigated only Se^{+4} gives rise to the formation of a yellow precipitate. Se^{+6} gives rise to a weak reaction (simultaneous presence of Se^{+4}?). A piazthiazole is formed with sulphur dioxide but it is colourless. Oxidising con-

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stituents must be eliminated otherwise they give rise to a violet oxidation product. Fe^{+3} also should be fixed by combination with KF. Mo^{+6} gives a pale blue tint such that at a concentration of 10^{-2} of Mo, Se can be detected only to 10^{-5}. Te^{+4} and Te^{+6} do not give the reaction. As already established by FEIGL[7], small quantities of Se can always be detected in commercial Te salts. About 0.01% of Se can be clearly recognised in a 10^{-2} solution of Te. All the other ions given in Table I were examined at a dilution of 100 : 1. They do not interfere with the reaction except in so far as their own colour acts deleteriously.

3. *Reagents*

2.5% diaminobenzidine hydrochloride in water.

We thank Mr PIEN, who so willingly placed a sample of diaminobenzidine at our disposal. We would express our best thanks also to Prof. Dr J. GILLIS who proposed us this investigation.

SUMMARY

First of all diaminobenzidine is described as a reagent for V^{+5}. It behaves in this respect, similarly to benzidine. On the other hand, with selenium there is formation of a piazselenole, which is insoluble in both acid and alkaline media and is intensely yellow coloured. Hence it is possible to detect Se^{+4} in this way in dilutions down to 1 : 10^6. At the same time the reaction is quite specific, but is interfered with by the presence of oxidizing constituents.

RÉSUMÉ

Tout d'abord, la diaminobenzidine est décrite comme réactif de V^{+5}. Elle se comporte comme la benzidine.

D'autre part, avec le sélénium, il y a formation d'un piazselenole insoluble et d'une coloration jaune, soit en milieu alcalin, soit en milieu acide.

Il est possible de déceler, à l'aide de ce réactif, le sélénium en dilution 1 : 10^6.

En plus, la réaction est tout à fait spécifique, mais elle est gênée par les oxydants.

ZUSAMMENFASSUNG

Zunächst wird Diaminobenzidin als Reagens auf V^{+5} beschrieben. Es verhält sich dabei ähnlich wie Benzidin. Andererseits tritt mit Selen die Bildung eines Piazselenols auf, das sowohl in sauren wie alkalischen Media unlöslich ist und intensiv gelb gefärbt ist. Dadurch ist es möglich Se^{+4} auf diese Weise in Verdünnungen bis zu 1 : 10^6 zu bestimmen. Gleichzeitig ist die Reaktion sehr spezifisch, wird aber durch die Anwesenheit oxydierender Bestandteile gestört.

REFERENCES

[1] Second Report: *Reagents for qualitative Inorganic Analysis*. New York–Amsterdam 1948.
[2] F. FEIGL, *Qualitative Analysis by Spot Tests*. New York–Amsterdam 1946.
[3] J. PIEN, *Le Lait*, 17 (1937) 673.
[4] A. A. NOYES, *Qualitative Chemical Analysis*. New York 1923.
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