2. Data, upon comparison with the work of Abramson with the same protein on quartz surfaces but by the cataphoresis method, show excellent agreement between the values of the ζ-potential obtained by the two methods between *P*H 3.8 and *P*H 5.2. Divergence of the values of ζ-potential beyond this range is explained by the differences in the concentration of the electrolyte utilized in the two experiments and probably, to some extent, by the differing effects of the ions used.

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DIPHENYLCARBAZIDE AS A TEST FOR CHROMIUM

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A search of the literature has revealed a number of organic reagents which have been used for the detection and estimation of chromium when present as chromate or dichromate. Of these organic compounds, diphenylcarbazide, $CO(NHNHC_6H_5)_2$, seemed very promising. Cazeneuve[1] used this reagent to detect chromic acid in a dilution of 1:1,000,000. Brandt[2] proposed the use of diphenylcarbazide as an inside indicator in the titration of iron with potassium dichromate, while more recently Snoddy[3] has made it the basis of a colorimetric method for estimating small amounts of chromium in fats. Scott[4] mentions the use of this reagent in detecting chromium as chromate.

Since the test is very simple to carry out and the reagent produces a distinctive violet or reddish-violet color with exceedingly small amounts of dichromate, it seemed desirable to compare the sensitivity of diphenylcarbazide with that of the ether—hydrogen peroxide test usually employed in qualitative analysis and to determine the best conditions under which the test might be applied.

Experimental Work

I. Tests with Potassium Dichromate

Solutions of potassium dichromate containing varying amounts of chromium were first tested with diphenylcarbazide and also by the ether–hydrogen peroxide method in order to determine which method was the more sensitive. Further, the tests with diphenylcarbazide were carried out in solutions acidified with different acids, namely, sulfuric acid, acetic acid and citric acid.

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[1] Cazeneuve, *Bull. soc. chim.*, [3] **23**, 592, 701, 769 (1900).

[2] Brandt, *Z. anal. Chem.*, **45**, 95 (1906).

[3] Snoddy, *J. Oil Fat Ind.*, **2**, 20 (1925).

[4] Scott, "Standard Methods of Chemical Analysis," D. Van Nostrand Co., New York. **1918**, p. 132.

The reagent was made by dissolving 0.2 g. of diphenylcarbazide in 10 cc. of glacial acetic acid and diluting to 100 cc. with 95% ethyl alcohol. The solution remains colorless for about one day, but thereafter gradually turns to a slightly reddish-brown color, even when kept in a brown bottle. This change in color, however, did not seem to render the reagent less sensitive.

The following procedure was used. A stock solution of potassium dichromate was made up to contain 10 mg. of chromium per cc. This solution was then diluted to a definite concentration as required and that volume pipetted out which gave four times the amount of chromium needed for the test at that particular dilution. This solution of potassium dichromate was treated with 4 cc. of 6 *N* ammonium hydroxide, diluted to 100 cc. and divided into four equal portions. One portion was acidified with 6 *N* sulfuric acid, an excess of about 2 cc. being added, followed by the addition of 3 to 5 cc. of ether and 3 cc. of 3% hydrogen peroxide solution. The mixture was shaken vigorously and the appearance of the ether layer noted. The three remaining portions were slightly acidified with sulfuric acid, acetic acid and citric acid, respectively, and 2 cc. of the diphenylcarbazide reagent was added to each. The solutions were allowed to stand for ten minutes before recording the formation of a red or reddish-violet color. When using 2 cc. of the reagent a deep red color is produced with relatively large amounts of dichromate while with small amounts of dichromate the solution assumes a distinctly violet color, the intensity of which varies with the amount of dichromate present. The 25cc. portions of solutions tested contained from 0.04 mg. to 0.00005 mg. of chromium, these concentrations representing 1 part in 625,000 and 500,000,000, respectively. A duplicate series of tests was carried out, the results of the two series being almost identical. It was found that the highest dilutions giving positive tests were as follows: for the ether–hydrogen peroxide method, 1 part of chromium in 1,250,000; for diphenylcarbazide, 1 part in 100,000,000 to 250,000,000 when using sulfuric acid, 1 part in over 71,000,000 when using acetic acid, and 1 part in 12,500,000 when in the presence of citric acid. These results indicate that diphenylcarbazide affords a much more sensitive test than does the ether–hydrogen peroxide method and that diphenylcarbazide is more sensitive when used in the presence of sulfuric acid than in the presence of either acetic or citric acid.

A series of blank tests was next run in duplicate on solutions containing 10 mg. of zinc, or 5 cc. of 3% hydrogen peroxide or both the zinc and hydrogen peroxide, and when acidified with 2 cc. of either 6 *N* sulfuric, hydrochloric or acetic acid. The final volume of each solution was between 25 and 30 cc. The zinc used was in the form of zinc nitrate. In every case a negative test was obtained upon adding 2 cc. of the reagent.

These blank tests were made on solutions containing zinc and hydrogen peroxide because these two substances might be present in a solution at the point of testing for chromium in the usual qualitative procedure.

Also, since the color produced by the reagent is probably due to oxidation by chromate or dichromate, it was thought that the nitrate ion in acid solution might itself cause a color change. The nitrate ion, of course, would normally be present in a solution at this point in the qualitative procedure if nitric acid were used to acidify the filtrate from the sodium hydroxide–sodium peroxide treatment preliminary to removing aluminum. Accordingly, three solutions were made as follows: (1) 25 cc. of water, 1 cc. of 6 N ammonium hydroxide and sulfuric acid until acid; (2) same as (1) but with 1 g. of sodium nitrate added; (3) same as (1) but with 1 g. of ammonium nitrate added. Two cc. of reagent was added to each solution. No color developed in any of the solutions.

II. Tests with Chromic Nitrate

The diphenylcarbazide reagent having been found to be very sensitive to dichromate ions, it was thought advisable to determine the sensitivity toward solutions obtained by starting with chromic ions and subjecting them to the usual Group III procedure of qualitative analysis. For this purpose chromic nitrate solutions containing definite amounts of chromium were used, while the procedure followed was that given by Reedy[5] for the analysis of Group III. After precipitating aluminum hydroxide, the filtrate (about 50 to 60 cc.) was cooled and divided into two equal portions. One part was acidified with acetic acid and 2 cc. of the diphenylcarbazide reagent added. The other portion, which would normally be used for the test for zinc, was tested for dichromate by the ether–hydrogen peroxide method already described under the tests with potassium dichromate. A comparison of the two tests for chromium was thus made from the same solution at the same time. It was found that the highest dilution giving a positive test with diphenylcarbazide was 1 part of chromium in approximately 1,666,000, while a dilution of 1 part in 250,000 failed to give a positive test by the ether–hydrogen peroxide method. Although the diphenylcarbazide reagent is again shown to be more sensitive than the ether–hydrogen peroxide method, it is to be noted that a smaller amount of chromium was detected when originally present as dichromate than when present as chromic ions and the latter put through the usual Group III procedure of qualitative analysis.

Another series of tests similar to those just described for chromic nitrate was carried out, but with 10 mg. each of aluminum, zinc and iron added to each solution tested. The aluminum was added in the form

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[5] Reedy, "Elementary Qualitative Analysis," McGraw-Hill Book Co., New York, 1924.

of the ammonium alum, the zinc as zinc nitrate, and the iron as ferric nitrate. The procedure was the same as when using chromic nitrate alone. The results obtained were almost the same as those obtained when using chromic nitrate alone, indicating that little or no chromium was lost during the precipitation and removal of other cations that might normally be present.[6]

It should be mentioned why, in the tests made on solutions originally containing chromic ions, acetic acid was used instead of sulfuric acid to acidify the solutions prior to adding diphenylcarbazide. In performing blank tests on the individual cations used in the various mixtures above, namely, aluminum, zinc and iron, several slight positive tests were obtained when using sulfuric acid to acidify the solutions before adding diphenylcarbazide, although the salts used were supposedly free from chromium. When the same blank tests were repeated, using acetic acid instead of sulfuric acid, no positive tests were obtained. The slight positive tests given when using sulfuric acid may have been due to very minute traces of chromium in the salts used, together with the fact that diphenylcarbazide is extremely sensitive when used in sulfuric acid solutions. Hence, acetic acid was to be preferred.

Acetic acid has the added advantage in that it is the acid used to acidify the solution prior to adding potassium ferrocyanide in making the test for zinc by this method. Thus the filtrate from the aluminum precipitation has only to be acidified with acetic acid, divided into two parts, one part treated with diphenylcarbazide to test for chromium and the other portion with potassium ferrocyanide for zinc.

The use of diphenylcarbazide possesses two other advantages.

1. It involves adding only one solution, whereas the ether–hydrogen peroxide method requires two. 2. It dispenses with the use of ether, which is usually regarded as a source of danger when used by beginning students in chemistry, due to its inflammability.

Summary

1. The sensitivity of diphenylcarbazide toward dichromate ions has been compared with the sensitivity of the ether–hydrogen peroxide method, the diphenylcarbazide proving to be much more sensitive.

2. The advantages offered by the use of diphenylcarbazide in place of ether and hydrogen peroxide have been pointed out.

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[6] The author gratefully acknowledges the assistance of Dr. R. B. Sandin, of the University of Alberta, who also carried out some of the analyses in order to eliminate the personal factor which might be involved in reading the various tests.