

7-Amino-3, 5-dimethyl-1, 2-benzisoxazole (II a).—Stannous chloride (14 g) was dissolved in conc. HCl (20 ml) by heating. To this clear solution, powdered 3, 5-dimethyl-7-nitro-1, 2-benzisoxazole (2 g) was added. The reaction mixture was then refluxed for 2 hrs. The crystalline amine hydrochloride was then dissolved in water, neutralised with dilute solution of ammonia and the precipitated free amine was extracted with ether. The amine was isolated by the evaporation of ether and crystallised from 50% alcohol. It melted at 91°. Yield 45%.

Similarly all other amines (II b-i) were prepared from the corresponding nitro compounds. The physical data of these amines are given in Table I.

7-(N⁴-Acetylsulphanilamido)-3, 5-dimethyl-1, 2-benzisoxazole (III a).—A mixture of 7-amino-3, 5-dimethyl-1, 2-benzisoxazole (0.01 mol), freshly prepared *p*-acetamidobenzene sulphonyl chloride (0.012 mol) and dry pyridine (10 ml) was heated on a water-bath for half an hour and kept at room temperature for 48 hours. It was then poured over crushed ice containing H₂SO₄. The precipitated solid was filtered, washed with water and crystallised from acetic acid. m.p. 242°. Yield 60%.

All other N⁴-acetylsulphanilamido compounds (III b-e) were also prepared as above.

3, 5-Dimethyl-7-sulphanilamido-1, 2-benzisoxazole (IV a).—7-(N⁴-Acetylsulphanilamido)-3, 5-dimethyl-1, 2-benzisoxazole (2 g) was refluxed with 50% hydrochloric acid (25 ml) and ethanol (10 ml) for 1 hr. The reaction mixture was filtered hot and diluted with ice-water. It was then neutralised with sodium bicarbonate solution and the resulting solid was filtered, washed with water and crystallised from 60% ethanol M.P. 225°. Yield 69%.

The same procedure was used for the preparation of all other sulphanilamido-1, 2-benzisoxazoles (IV b-e).

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A STAINING TECHNIQUE FOR MICROSCOPIC IDENTIFICATION OF BOEHMITE (GAMMA—ALOOH)

BOEHMITE (gamma—ALOOH) usually occurs in nature in a very fine-grained form. It is a common constituent of most of the bauxite deposits. It is more abundant in the Karst bauxites and the pisolitic varieties of lateritic bauxites than in the massive bauxites¹. While the other two important allitic minerals of bauxites, namely gibbsite and diasporite are usually well crystallised and lend themselves readily to optical methods of identification, boehmite owing to its sub-microscopic character often poses problems during routine petrographic examination of bauxites. A staining technique has been developed for rapid identification of boehmite both in thin section and grain mounts.

The staining method reported here is actually a modification of the staining tests employed for identification of clay minerals². During the detailed petrological studies of the bauxite samples from the East Coast of India, it was found that besides the clay minerals, boehmite also picks-up the aniline dyes used for staining clay minerals. Since there is a considerable difference in the refractive indices of boehmite and the common clay minerals, it is not difficult to differentiate them in spite of their poorly crystallised nature.

Reagents

Solvents : Nitrobenzene, Methylene iodide.

Dyes : Safranin-Y, Malachite green, Crystal violet.

Nitrobenzene and methylene iodide are mixed in suitable proportions such that the resulting solution has a refractive index of about 1.62. The staining solutions are prepared in separate glass containers by dissolving about 50 mg of the dyes in about 25 ml of the nitrobenzene-methylene iodide solution. The staining solutions will last for several weeks if they are refrigerated.

The thin section to be stained is left uncovered after grinding it to about 30 microns thickness. The surface of the section is washed free of Canada balsam using xylol. A few drops of 1 : 1 HCl are smeared on the surface of the thin section and

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allowed to stand for about five minutes. The surface is washed free of acid with distilled water and dried at room temperature by blowing dry air over the slide. One or two drops of the staining solution are spread over the thin section, kept undisturbed for about three minutes and then observed under the microscope. Boehmite shows the following colouration due to the absorption of the dye [Figs. 1 (a) and 1 (b)].

Safranin-Y .. Deep pink .
Malachite green .. Bluish green
Crystal violet .. Blue purple.

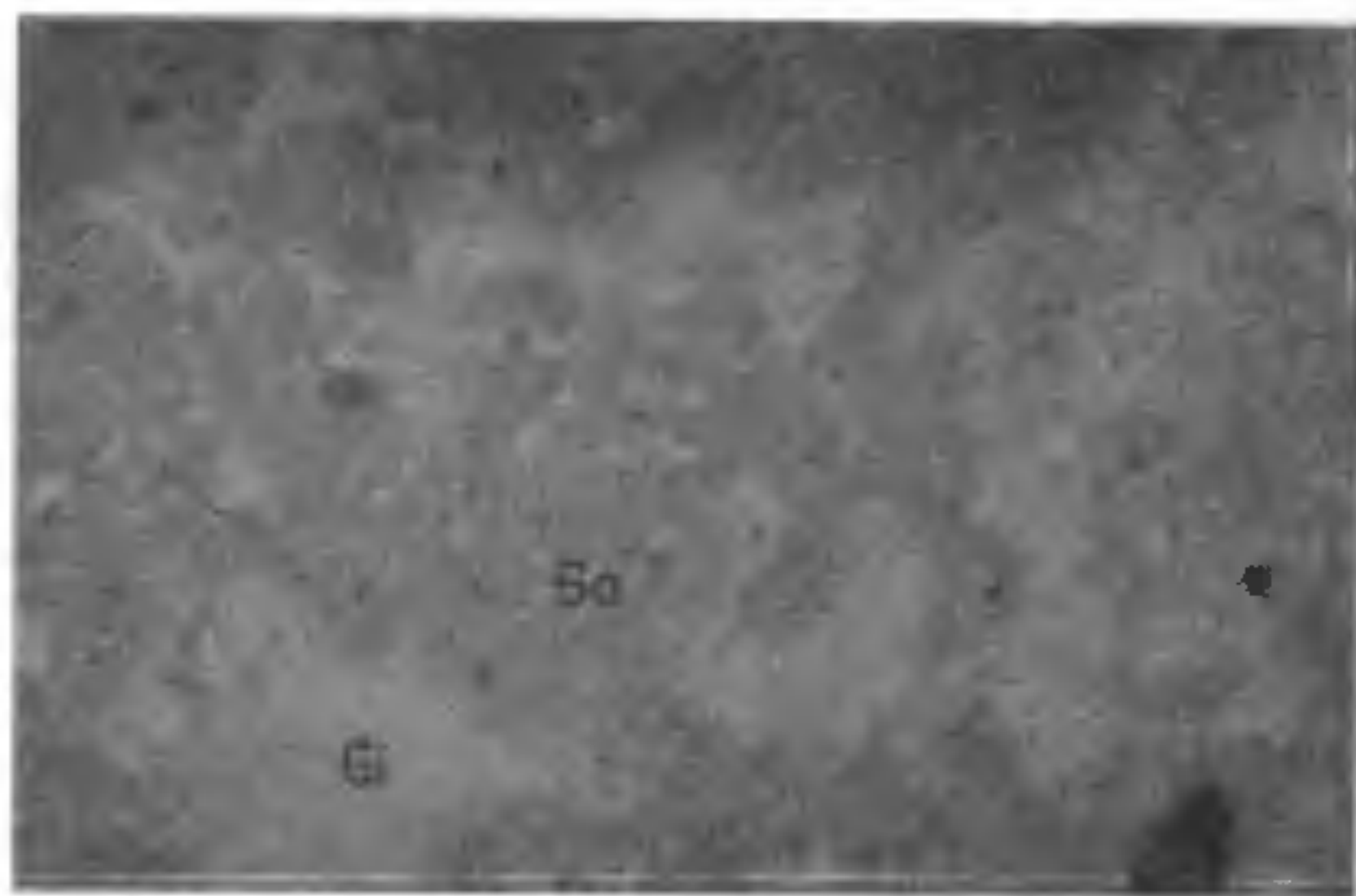


FIG. 1 (a). Bauxite before staining, boehmite (Bo), gibbsite (Gi), plain light (X 40).

The results of these staining tests are somewhat similar to those obtained with kaolinite but with

slight changes in the hues of the colours. It may be difficult to distinguish between boehmite and basal sections of kaolinite in a thin section even after staining. This can be resolved by staining the powdered sample as follows.

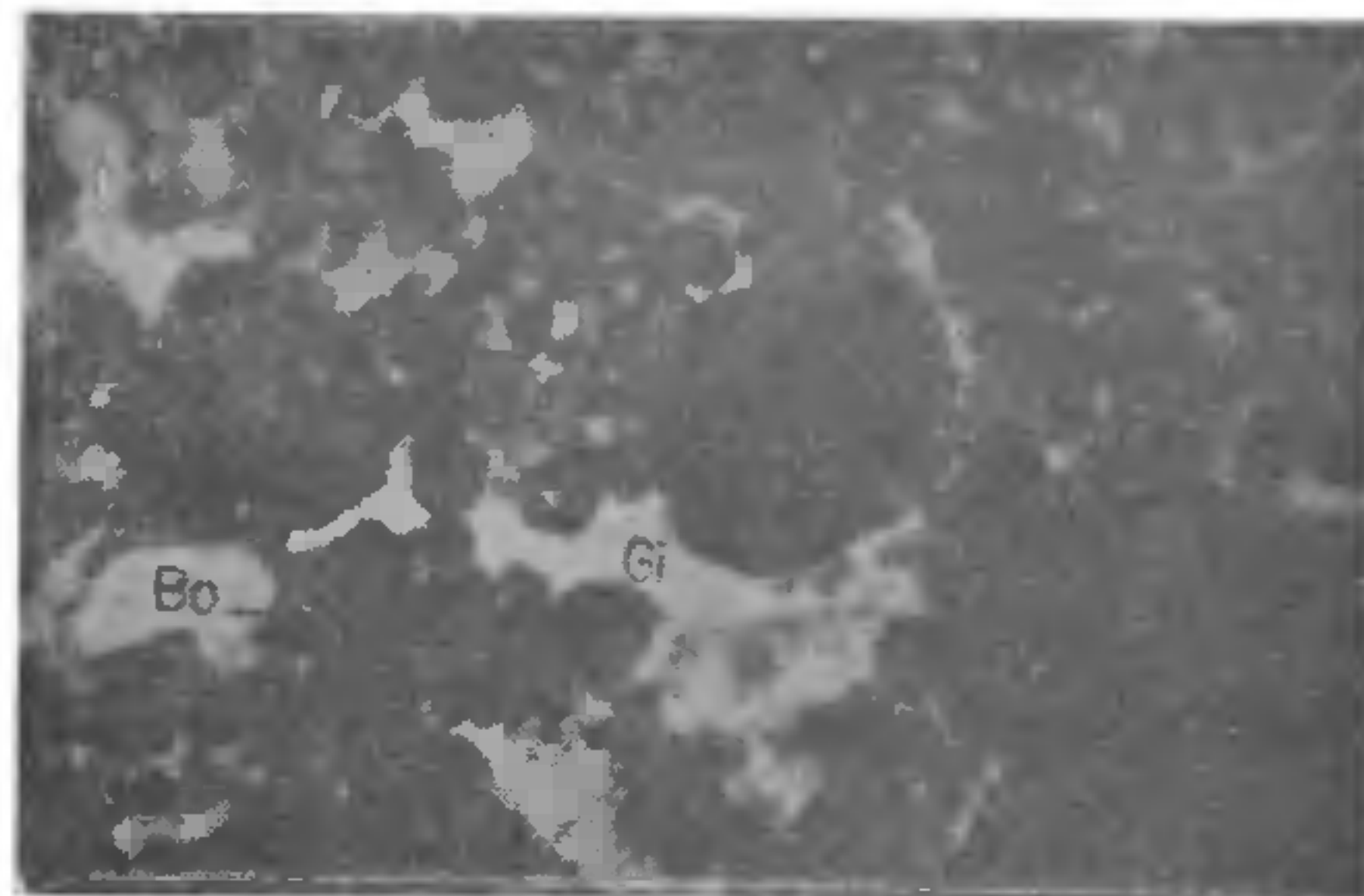


FIG. 1 (b). Bauxite after staining with Malachite green, plain light (X 40).

The sample is crushed to pass 100 BSS mesh sieve. The -150, +200 BSS mesh material is suitable for microscopic examination. A few milligrams of the -150, +200 mesh material is kept immersed in warm 1 : 1 HCl in a Corning beaker for about 30 minutes. The supernatant acid is decanted and the grains are washed free of acid with distilled water and dried over a sand bath. A small amount of the acid treated powder is placed on a glass slide, a drop of the staining

TABLE I

Optical properties of the transparent bauxitic minerals immersed in staining solutions

Mineral	Relief in the medium ($n = 1.62$)	Optical character	Colour of the mineral after staining		
			Safranin-Y	Malachite green	Crystal violet
1. Gibbsite	Negative	Biax. +	N.S.	N.S.	N.S.
2. Boehmite	Positive	Isotropic, rarely anisotropic	Deep pink	Bluish green	Blue purple
3. Diaspore	Positive	Biax. +	N.S.	N.S.	N.S.
4. Kaolinite	Negative	Isotropic to Pleochroic	Deep pink to Yellow red	Pale sea green to Ink blue	Blue purple to Deep pink
5. Montmorillonite	Negative	Isotropic or Biax. -	N.S.	N.S.	N.S.
6. Quartz	Negative	Uniax. +	N.S.	N.S.	N.S.

(N.S. = Not stained).

solution added to it, thoroughly mixed with a clean glass rod and allowed to stand for a minute. Boehmite and kaolinite absorb the dyes and are similarly coloured, but boehmite shows a positive relief and kaolinite a negative relief in the immersion medium used, which can be readily ascertained by the movement of the Becke line. Boehmitic portions in boehmite-gibbsite or boehmite-diaspore intergrowths may easily be distinguished after staining [Figs. 2(a) and 2(b)]. The transparent minerals commonly found in bauxites may be identified by using the characteristics shown in Table I.

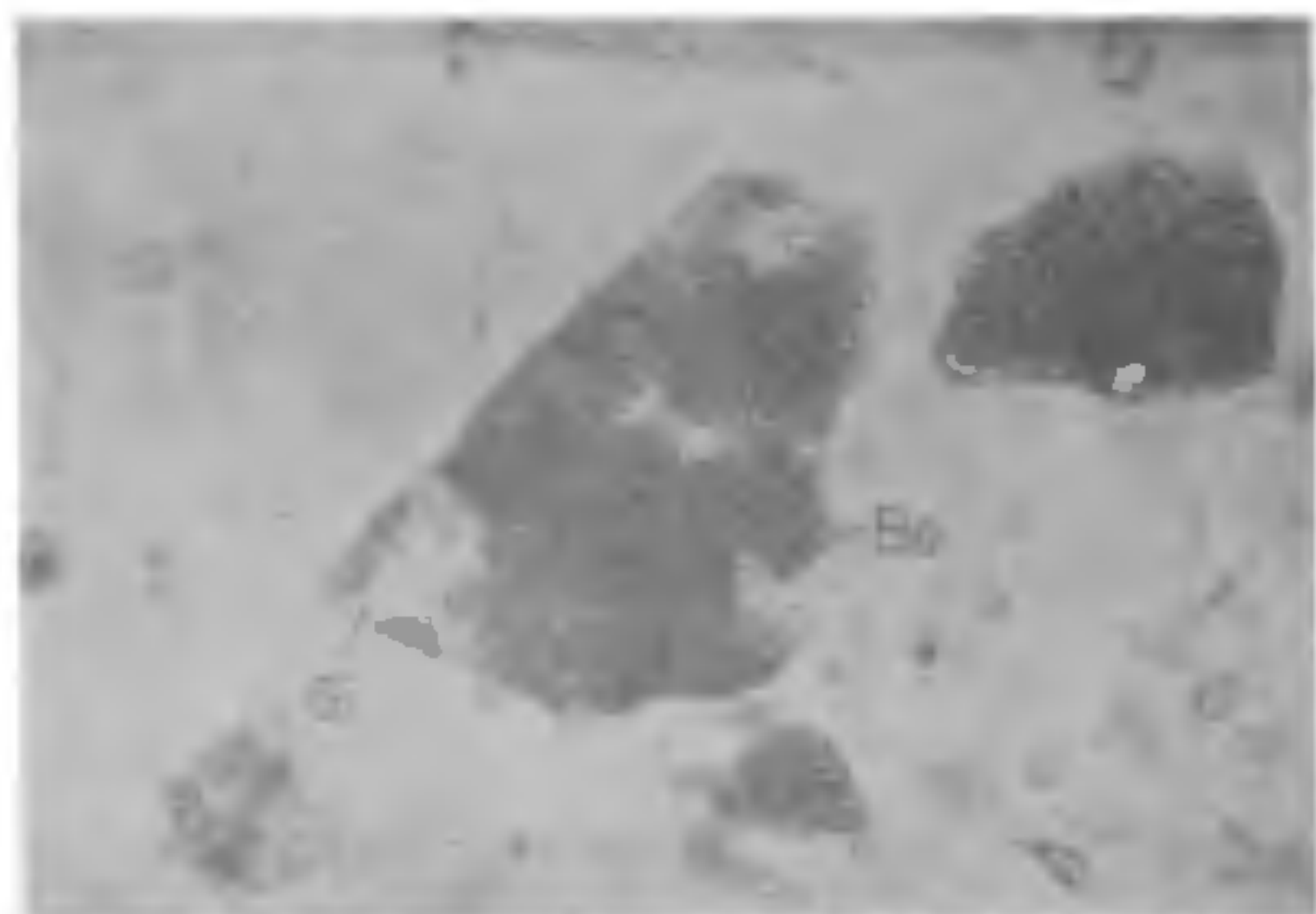


FIG. 2(a). Boehmite-gibbsite intergrowth stained with Malachite green, plain light ($\times 40$).

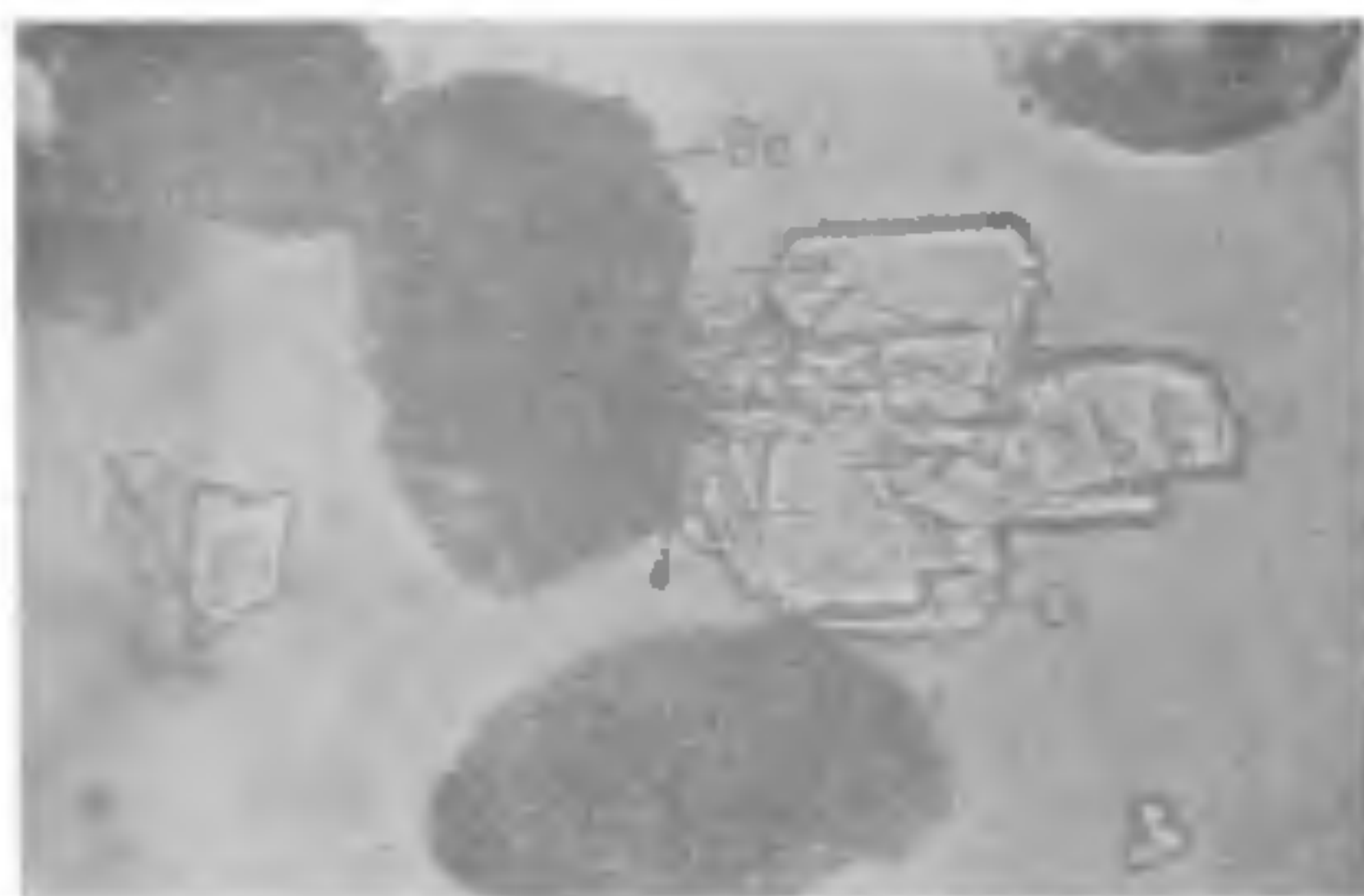


FIG. 2(b). Boehmite and diaspore (Di) grains stained with Malachite green, plain light ($\times 40$).

The colour test employing *p*-aminophenol may be used to confirm the presence of montmorillonoids³. *p*-aminophenol did not give a distinctive colour test in the case of boehmite samples used in this study.

In the presence of kaolinite on staining with safranin-Y boehmite takes on a brownish pink to yellowish brown colour compared to deep pink colour of kaolinite. However, staining with Malachite green produces distinctly different colours in the two minerals as shown in Table I, and hence appears to

be best suited for differentiating between boehmite and kaolinite. The identification of kaolinite, gibbsite, boehmite and diaspore used in these tests was confirmed by X-ray diffraction analysis; montmorillonite by the *p*-aminophenol colour test and quartz by optical methods.

The staining technique reported here has the following advantages—(1) It is a rapid and cheap method of identification of the transparent bauxitic minerals, (2) It helps in distinguishing between boehmite and crypto-crystalline gibbsite/diaspore during modal analysis and (3) It is possible to detect the presence of boehmite even when it is present in amounts less than the detection limits⁴ of Derivatography (0.3%) and X-ray diffraction (0.5%) by microscopic observation of the stained powdered sample.

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VARIATIONS IN TISSUE GLYCOGEN CONTENT SERUM LACTATE AND GLUCOSE LEVELS DUE TO COPPER INTOXICATION IN THREE FRESHWATER TELEOSTS

COPPER is widely used as an algacide and in the treatment of disease and parasitism in fishes¹. The latter application sometimes poses the problem of toxicity to the fish treated. In the present investigation the author has studied the effect of copper sulphate (5, 10 and 15 ppm) on glycogen content in liver, muscle, brain and kidney tissues and the lactate and glucose levels in serum of *Labeo rohita* (Ham.), *Ophicephalus punctatus* (Bloch.) and *Clarias batrachus* (Linn.).

Twenty-four live *L. rohita*, *O. punctatus* and *C. batrachus* (18–20 cm) were acclimatized in the laboratory for 3–4 days. A group of six fishes (*L. rohita*, *O. punctatus* and *C. batrachus* each) were placed in a glass aquaria containing 5, 10 and 15 ppm of copper