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Note

Rapid, sensitive method for the separation and detection of arsenic compounds in biological systems

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Arsenate, arsenite and their methylated derivatives methylarsonic acid and cacodylic acid are released into the environment in large quantities as industrial by-products or as fungicides, herbicides, and insecticides. A simple qualitative procedure for monitoring their presence in the environment has not been reported. A number of isolation systems have been used but they require either time-consuming sample preparation or multiple chromatographic systems[1–4]. Often the compounds are difficult to visualize. In this paper we describe a simple, rapid, one-step procedure for identifying arsenic compounds in complex biological systems.

EXPERIMENTAL

Sample preparation

All chemicals used were analytical grade. Sodium arsenate, sodium arsenite, sodium methyl arsonate and sodium cacodylate were prepared in distilled water or in domestic sewage sludge obtained from an anaerobic sewage digester. In the latter case, particulate matter was removed from the sample after the addition of the arsenical by centrifugation at 2500 × *g* for 5 min. The supernatant contained the free, soluble arsenic derivatives. If non-covalently bound arsenic present in cellular material was to be determined, the particulate matter was boiled for 10 min with water or water–methanol (60:40). After boiling, the sample was centrifuged. Insoluble arsenic salts were solubilized by mixing the sludge with an excess of Dowex 50 Na^+. Following centrifugation, the supernatant was adjusted to pH 7.0.

Electrophoresis

Electrophoresis was performed on non-fluorescent cellulose thin-layer chromatographic sheets (20 × 20 cm) from Machery-Nagel and Co. (Düren, G.F.R.) or Eastman-Kodak (Rochester, N.Y., U.S.A.). Prior to use the chromatography sheets were washed with distilled water and then dried. Washing was necessary to remove an unknown material which sometimes interfered with subsequent color development of the electrophoretogram. The cellulose absorbed a contaminant from the laboratory air, and it was therefore necessary to use the sheets within a few hours after washing.

Samples were usually spotted 8 cm from the end of the cellulose sheet. This

permitted the maximum separation of the compounds migrating to the anode. When spotting large amounts of biological sample, there was often a considerable build-up of material in and at the edge of the spot. This did not affect the migration of the arsenic compounds provided the area was thoroughly wetted prior to electrophoresis. However, if this precaution was not taken, streaking and poor resolution occurred.

Electrophoretic migration was conducted on a Desaga (Heidelberg, G.F.R.) electrophorator in 0.05 *M* sodium citrate buffer, pH 7.0. A voltage of 400 mV was applied to the paper for 60 min.

Identification

After electrophoresis the sheet was air-dried and placed in a 3-l chromatography chamber together with a small beaker containing 10 ml of ammonium sulfide. After 30-min exposure arsenite was visible as a bright yellow spot. The sheet was then sprayed with Jüngnickel's Reagent A[5] until it was saturated. Methylarsonic acid appeared as a light blue spot. The sheet was then exposed to short-wave UV radiation for 20–30 min. Arsenate appeared as a dark blue spot and cacodylate as a lilac pink spot. The background blue color was removed by exposing the plate to concentrated ammonium hydroxide fumes.

RESULTS AND DISCUSSION

As shown in Fig. 1, arsenate, methylarsonate and cacodylate migrate to the anode at pH 7.0. Arsenite moves toward the cathode. Assigning arsenate a mobility of 1.0, methylarsonate and cacodylate have relative mobilities of 0.86 and 0.47, respectively. The compounds are well separated, but identification is made even easier by the sequential nature of color development and the difference in color, *i.e.* the light blue methylarsonate appears before the dark blue arsenate is seen.

Table I summarizes the color development scheme, indicating the color and

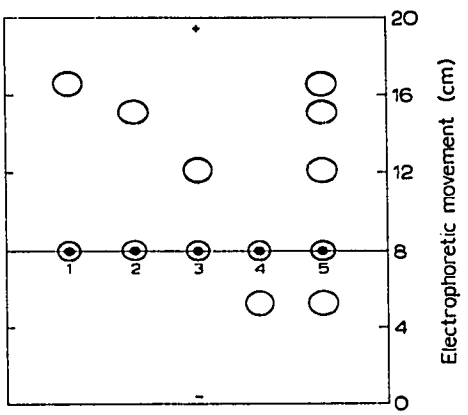

Fig. 1. Electrophoretic separation of arsenic compounds. Samples were spotted on 20 × 20 cm cellulose sheets. Spots shown are: (1) sodium arsenate, (2) sodium methylarsonate, (3) sodium cacodylate, (4) sodium arsenite, and (5) a mixture of the preceding four compounds. The compounds were electrophoresed in 0.05 *M* sodium citrate buffer, pH 7.0, for 60 min, and developed sequentially with ammonium sulfide, Jüngnickel's Reagent A, and short-wave UV radiation.

TABLE I

CHARACTERISTICS OF THE DETECTION SYSTEM

Compounds (in order of appearance)	*Color*	*Treatment*	*Minimum quantity detectable**	*Mobilities relative to arsenate*
Arsenite	Yellow	$(NH_4)_2S$	0.5	—
Methylarsonate	Blue	Jüngnickel's reagent	1.5	0.86
Cacodylate	Lilac-pink	UV	2.0	0.47
Arsenate	Blue	UV	0.10	1.0

* Micrograms spotted in 1.2-cm-diameter circle.

the time of appearance of spots. Also shown are the minimum detection limits. These are calculated as the minimum amount of compound which can be readily identified in a spot 1.2 cm in diameter. These sensitivities compare favorably with those reported for other systems[1–4].

Sodium phosphate, sodium tellurate, sodium nitrate, sodium nitrite, sodium sulfite and sodium sulfate were tested in the system to determine if they would interfere with the identification of the arsenic compounds. With the exception of phosphate, 0.5 mg of these compounds did not interfere with migration nor did they form colored complexes with the developing reagents. Phosphate appeared as a blue spot when exposed to UV radiation. However, it had a relative mobility of 0.84, was well separated from arsenate, and appeared after methylarsonate.

The method described for the determination of the four arsenic derivatives is simple, rapid, sensitive and inexpensive. Color development is sequential and specific. Other salts present in natural systems do not interfere with color development or electrophoretic mobility. In the natural system we have used to test this procedure large quantities of contaminating salt, protein, lipid, and carbohydrate had no adverse effects. The system should be valuable for the qualitative analysis of arsenic in natural systems.

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