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A Simple Method of Arsenic Speciation

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Arsenic is known for its biological toxicity (FEINGLASS 1973). Arsenate [As(V)] uncouples oxidative phosphorylation reactions (LEHNINGER 1972) and competes directly with phosphate during microbial (BUTTON et al. 1973) and tissue cell (HILBORN 1976) phosphate transport. Arsenite [As(III)] is also toxic but most likely reacts differently than As(V) in biological systems. For example, As(III) has been shown to affect microbial growth by inhibiting a recombination repair dependent step in the repair of damaged DNA (ROSSMAN et al. 1975). Methylated forms of As(III) are even more biologically toxic than As(V) or As(III) (WOOD 1974). The exact mechanism of all forms of acute arsenic poisoning, however, is still not clear. Perhaps even more significant from an environmental standpoint is that the long term effects of exposure to low levels of arsenic are not known, particularly now that there is indirect evidence that arsenic may be a human carcinogen (SUNDERMAN 1975).

Analytical difficulty in speciation of arsenic at low levels (part per billion concentrations) in water, soils, sediments, and biological tissues has contributed to the present lack of understanding. The most sensitive methods for arsenic detection (atomic absorption; EDIGER 1975 and distillation AMERICAN PUBLIC HEALTH ASSOCIATION 1971) require transformation of the arsenic to a single chemical form so that only total arsenic can be quantified. Potentially useful atomic absorption methods of arsenic speciation are discussed in the work of CLEMENT AND FAUST (1973).

In this study we have used a modification of the phosphate-detection procedure (MURPHY and RILEY 1962) to differentiate between dissolved As(V) and dissolved As(III) in water samples. This simple method is based on the fact that arsenate, like phosphate and silicate, forms an alcohol extractable molybdate complex (blue colored when reduced) while arsenite does not (JOHNSON 1971). The results show that this procedure used prior to total arsenic analysis will enable speciation of arsenic to the detection limits of the common methods used for total arsenic analysis.

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MATERIALS AND METHODS

As(V) ($Na_2HAsO_4 \cdot 7H_2O$) and As(III) ($NaAsO_2$) standards were made by serial dilutions of stock solutions containing 20 micrograms (µg) As per milliliter (ml) of distilled water. The usual MURPHY and RILEY (1962) phosphate reagent was used (ammonium molybdate, 0.9 g/l; potassium antimony tartarate, 0.02 g/l; ascorbic acid, 0.02 g/l) except that the final concentration of H_2SO_4 in the reagent-sample mixture was 0.5N instead of 0.4N. For spectrophotometric assays, 10 ml of reagent were added to 40 ml of each standard as contained in carefully washed 50 ml volumetric reaction flasks. The arseno-molybdate complex (blue color) was allowed to develop for 90 minutes at room temperature (JOHNSON 1971). The absorbance of an aliquot was measured at an optimized wavelength of 712 nanometers in a spectrophotometer cell with a path length of one centimeter. Six ml of isoamyl alcohol were then added to a 4 ml aliquot of the sample and the mixture was shaken vigorously. The absorbance of both the alcohol phase (one extraction) and the aqueous phase was measured.

For the isotopic assays, carrier-free $^{74}As(V)$ was added to quadruplicate standard solutions of arsenate as contained in a 50 ml volumetric reaction flask. Five ml of sulfide reducing agent (JOHNSON 1971) were added to duplicates of each standard to reduce $^{74}As(V)$ to $^{74}As(III)$. After 15 minutes, 10 ml of the modified MURPHY and RILEY (1962) reagent were added to both the reduced and non-reduced standard solutions. The arseno-molybdate complex was then allowed to develop for 90 minutes. Two ml aliquots of each standard were placed in glass or polystyrene test tubes and extracted 3-5 times with 0.5 ml water saturated isoamyl alcohol. After the last extraction 1 ml of ethanol was added to the 2 ml aliquot to ensure complete recovery of the radioactivity in the aqueous phase. Each extract (alcohol phase) and the 2 ml aliquot with ethanol wash (aqueous phase) were added to separate vials containing 10 ml of toluene Triton-X 100 scintillation cocktail. The radioactivity in each sample was then determined by liquid scintillation spectrometry.

RESULTS AND DISCUSSION

Table 1 shows that as expected dissolved As(V) will form a blue-colored arseno-molybdate complex while dissolved As(III) does not. This fact is the

basis for the reduction methods developed to eliminate arsenate interference in the detection of phosphate in soils (VON SCHOUWENBURG and WALINGA 1967) and natural waters (JOHNSON 1971). Table 1 also shows that the blue arseno-molybdate complex can be extracted with isoamyl alcohol in the presence of 0.5N H_2SO_4. However, the results in Table 1 do not ensure that dissolved As (III) is completely isoamyl alcohol insoluble or that the As(V) derived compound is completely isoamyl alcohol soluble.

TABLE 1
Extraction of Arseno-molybdate Complex with Isoamyl Alcohol as Measured by Absorbance

As(V) concentration	Absorbance*		
	H_2O-phase before extraction	Alcohol phase (1 extraction)	H_2O-phase after extraction
µgAs/50 ml	milli-o.d.	milli-o.d.	milli-o.d.
0	3	2	--
20	60	56	12
40	141	120	18
60	240	185	34
100	290	242	15
100**	3	4	--

The radioisotope studies were done to examine these possibilities. The results in Fig. 1 show that after 3 extractions As(V) (non-reduced samples) can be essentially completely removed from the aqueous phase (clear areas of bar-graph) and that As(III) (reduced samples) remains predominantly in the aqueous phase (hatched areas of bar graph). The relatively constant 4% As(III)-alcohol phase carryover with each extraction (Fig. 1) was indicative of arsenic partitioning between the alcohol and aqueous phases.

*Mean of duplicate values corrected for blank.
**Contained 100 µg As(III)/50 ml ($NaAsO_2$).

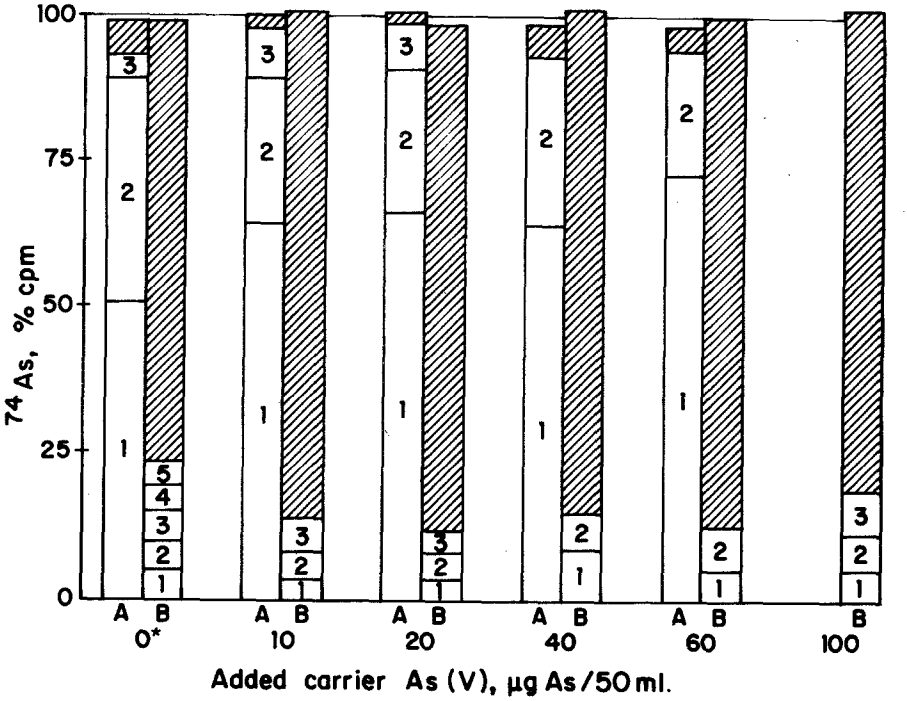

FIGURE 1. ^{74}As-molybdate Extractions of Non-reduced (A) and Reduced (B) Standard Solutions. Hatch Marks Represent % cpm in Aqueous Phase and the Clear Areas Represent % cpm in Each (numbered) Isoamyl Alcohol Extract (Mean Values of Duplicates).

Table 2 shows the effect of increasing isoamyl alcohol concentration on water solubility of As(III). The As(III) partition ratios for several alcohol:water systems reflect the slight solubility of arsenite in isoamyl alcohol. Thus while three alcohol extractions are recommended for As(V) derived arseno-molybdate removal from the aqueous phase, the alcohol addition should never exceed 25% of the sample volume. Because As(III) is slightly soluble in isoamyl alcohol, carry-over of As(III) in each alcohol fraction must be corrected for according to the partition ratios given in Table 2.

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*Reaction conducted in a polystyrene vessel.

TABLE 2
Partitioning of As(III) Between
Isoamyl Alcohol and Water*

Alcohol:Water ratio of sample	Alcohol phase As(III) in extraction no.: 1	2	3	Dissolved As(III) alcohol:water partition ratio
	100(cpm extracted/remaining)			
2:1	22.3	21.6	21.4	1:4.5
1:1	11.7	10.5	11.1	1:9
1:2	5.8	5.5	5.7	1:18
1:4	4.0	3.1	2.5	1:36

As with phosphate (RYDEN et al. 1972) arsenate sorption to glassware must also be considered at low concentrations. When carrier-free As(V) was reacted with molybdate reagent in acid washed glass tubes, only 35% could be removed in three alcohol extractions while 93% was removed when the reaction was carried out in polystyrene tubes (Fig. 1). Thus specially treated glassware or plastic ware must be used for samples containing low levels of As(V).

The implication of these results is that low concentrations of As(V) can easily and routinely be separated from As(III) in water, soil, sediment and biological samples using procedures analogous to well established phosphorus methodology (STRICKLAND and PARSONS 1968). Once separated total As(III) or As(V) can then be determined by atomic absorption or distillation allowing arsenic speciation at part per billion levels.

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*Each sample contained $^{74}As(V)$ with 100 µg As(V)/l carrier before reduction to As(III) by methods described in the text.

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