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Simple, Fast, and Sensitive Method for Quantification of Tellurite in Culture Media▽

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A fast, simple, and reliable chemical method for tellurite quantification is described. The procedure is based on the $NaBH_4$-mediated reduction of TeO_3^{2-} followed by the spectrophotometric determination of elemental tellurium in solution. The method is highly reproducible, is stable at different pH values, and exhibits linearity over a broad range of tellurite concentrations.

The tellurium oxyanion tellurite is toxic for most organisms, making important its accurate assessment. Several methods for quantifying tellurite have been described to date. However, most of them are rather complicated and require sophisticated equipment and in some cases the detection is not quite sensitive enough to allow the assessment of TeO_3^{2-} concentrations below 50 μg/ml (200 μM). For example, the analytical determination of tellurium (Te) oxyanions by atomic absorption spectrometry (AAS) is hampered by poor sensitivity. Where flame or electrothermal AAS routinely yields detection limits of less than 10 ppb for iron (16), normal flame AAS tellurium detection limits are 100 to 1,000 times higher and require pretreatment to achieve the +IV oxidation state before analysis (11).

On the other hand, hydride generation AAS (HGAAS) is used to achieve ppb-level detection limits for Se and Te as well as arsenic and antimony among others. For Te the volatile hydride gas H_2Te is generated by first converting the metalloid to the +IV oxidation state and then by chemical reduction to the gaseous hydride using—almost universally—sodium borohydride ($NaBH_4$). In automated HGAAS systems, an inert purge gas sweeps the volatile hydride formed in a glass reaction vessel into a quartz cell heated by the AAS flame where gaseous hydride decomposition and atomization occur. Though tellurite reduction, precipitation, and detection methods have been reported (3, 17), they are temporally relatively unstable and pH dependent.

Since tellurium is toxic and environmentally important (7, 8), determining low concentrations in bacterial cultures is very desirable and a simple analysis without pretreatment steps that could quickly establish total metalloid oxyanion content in a liquid sample would be a plus. Here we report a new method for the determination of tellurite in bacterial culture media. This procedure is based on the $NaBH_4$ reduction of tellurite to the elemental form, which is analyzed spectrophotometrically at 500 nm or 320 nm (see below), by which the light scattered by the particles of elemental metalloid in solution is measured.

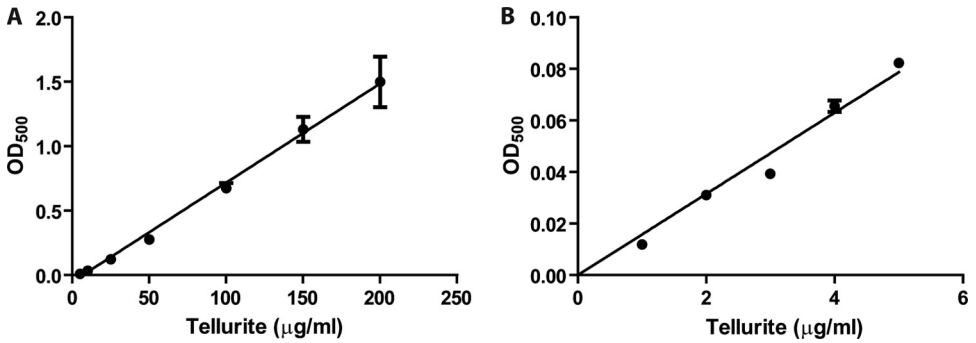

FIG. 1. Calibration curves to determine K_2TeO_3 concentrations in LB (A) (R^2 = 0.9963) or M9 minimal (B) (R^2 = 0.9740) medium. Optical density at 500 nm was determined after reducing the oxyanion with sodium borohydride. Error bars denote 1 standard deviation of three replicates.

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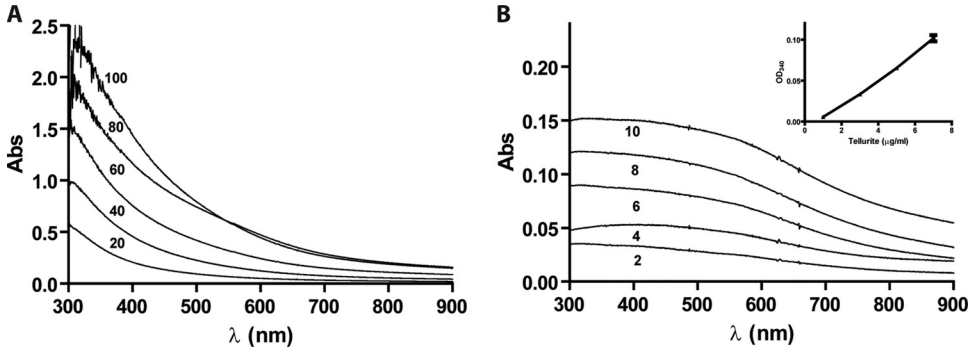

FIG. 2. Absorption spectra after reducing samples of LB (A) or M9 (B) culture medium containing increasing tellurite concentrations with 3.5 mM $NaBH_4$. Tellurite concentrations used were 20, 40, 60, 80, and 100 (LB) and 2, 4, 6, 8, and 10 (M9) μg/ml. (Inset) Calibration curve in M9 medium using the absorbance maxima at 320 nm.

While the detection limits do not compare to those of HGAAS (14) or capillary electrophoresis (13), they do approach those of old flame AAS but involve a much simpler and quicker procedure requiring only one reagent and a spectrophotometer to determine total content of solutions of +IV oxyanions in solution. Linear calibration range, method development time and probe stability, effect of sample pH, common interferences, and detection limits were investigated.

Calibration curves to determine K_2TeO_3 concentrations in routinely used microbiological culture media such as Luria-Bertani (LB) or M9 minimal medium amended with 0.2% glucose (15) were constructed. A set of solutions containing increasing concentrations of K_2TeO_3 (Sigma) were prepared in LB or M9 culture medium, and the tellurium oxyanion was quantitatively reduced using freshly prepared 3.5 mM $NaBH_4$ (final concentration).The reaction was carried out at 60°C for 10 min (bubbling was overcome by vortexing), and after 5 min at room temperature, the optical density at 500 nm (OD_{500}) was determined spectrophotometrically as described previously (4, 5, 9, 12). Blanks contained no borohydride. Figure 1 shows that in both media good curve linearity was obtained, with r^2 values of 0.9740 and 0.9963 for LB and M9, respectively. Tellurite concentrations lower than 1 μg/ml or higher than 200 μg/ml were also tested, but OD_{500} values were close to the spectrophotometer error limit at low concentrations or nonlinear above 200 μg/ml (not shown). Thus, the $NaBH_4$ method allows determination of a wide range of tellurite concentrations in a fast and simple way. Tellurite concentrations lower than 50 μg/ml in both rich and minimal media can be easily determined; the experimental error was about 10%, similar to that reported for the diethyl dithiocarbamate (DDTC) tellurite method (17).

To analyze the resulting solutions after tellurite reduction by $NaBH_4$, absorption/scattering spectra were determined. Figure 2 shows that spectra from LB and those from M9 after tellurite reduction are quite different, which may be a consequence of the different chemical compositions of these culture media. In both cases, absorption spectra showed linearity between optical density at 500 nm and tellurite concentration in the sample. However, high tellurite concentrations (~100 μg/ml) caused a loss of linearity in LB medium.

Figure 2B shows that in M9 medium there is a zone around 320 nm exhibiting higher optical density than that at 500 nm, which represents an advantage in the determination of tellurite in chemically defined culture media. This is reflected in a wider range of measurable concentrations at 320 nm (Fig. 2B, inset), as well as in a higher sensitivity of the method as determined by the slope of the calibration curve. The product of tellurite reduction by $NaBH_4$ showed good stability at both wavelengths in rich and minimal culture media (not shown).

Since in M9 medium the method allows the determination of minor tellurite concentrations (1 to 20 μg/ml), it would be of great help in assessing tellurite uptake in tellurite-sensitive microorganisms whose MICs range from 1 to 10 μg/ml. Sulfur-containing salts, commonly present in culture media as sulfites and sulfates, did not interfere with our $NaBH_4$ method for tellurite assessment at concentrations up to 0.5 M (not shown).

As shown in Fig. 3, tellurite assessment was not affected by

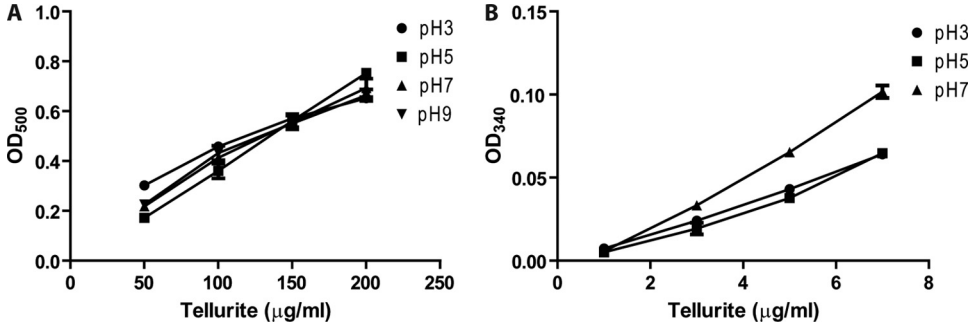

FIG. 3. Effect of pH in determining tellurite concentrations in LB (A) and M9 minimal (B) media.

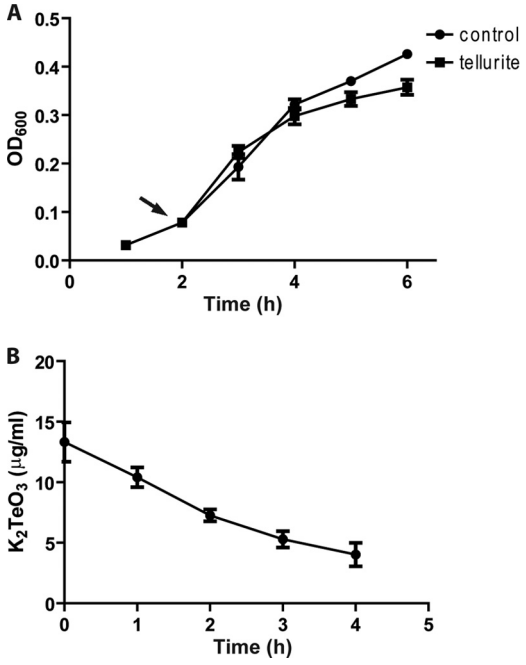

FIG. 4. Tellurite uptake by *Escherichia coli*. Time zero in panel B represents the moment of tellurite addition.

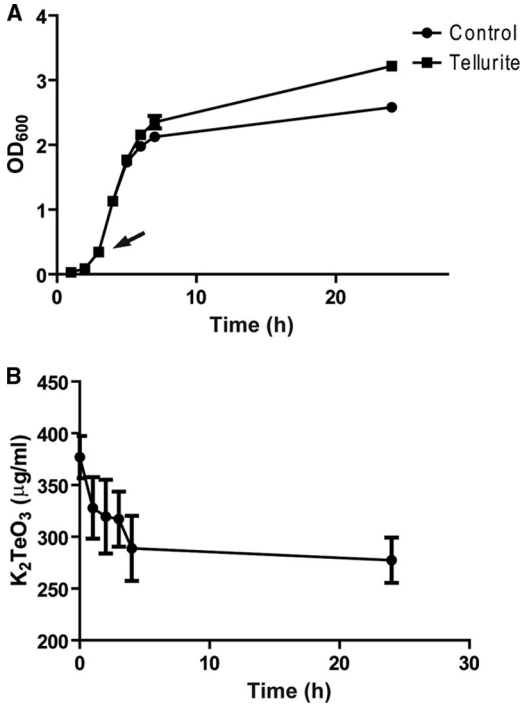

FIG. 5. Tellurite uptake by *Aeromonas caviae* ST. See the text for details.

the pH of the culture medium. In fact, linearity was observed in a wide pH range with minor slope changes in LB. Similar results were obtained with M9 medium, although tellurite assessment was not possible at pH values higher than 7.0 because of the formation of a precipitate. This may be due to an interaction of the phosphate salts present in the medium and some charged (2^+) chemical species forming at alkaline pH values, as has been reported earlier (17).

To date, the most commonly used procedure for determining tellurite in culture media is that involving the spectrophotometric determination (340 nm) of the complex that forms between tellurite and diethyl dithiocarbamate (17). This procedure has been used to assess tellurite uptake by the phototrophic bacterium *Rhodobacter capsulatus*, which is naturally resistant to K_2TeO_3 (MIC, ~1.4 mM) (2, 3). However, K_2TeO_3 uptake studies in highly sensitive cells such as *Escherichia coli* (MIC, ~4 μM) are difficult to carry out because of the low concentrations of toxicant present in the culture medium, far below the detection limit of the DDTC procedure (17).

In this context and for testing the applicability of our method *in vivo*, we used the tellurite-sensitive bacterium *E. coli* BW25113 (10) and the tellurite-resistant *Aeromonas caviae* ST (5, 6). An overnight culture of *E. coli* BW25113 in M9 minimal medium was diluted 100-fold with fresh M9 supplemented with 0.2% glucose and grown at 37°C with shaking. When the OD_{600} was 0.1, the culture was amended with 20 μg/ml K_2TeO_3 (arrow, Fig. 4A). Then aliquots were taken at the indicated times and cells were centrifuged at 8,500 × *g* for 3 min; supernatants were used to assess extracellular tellurite by our $NaBH_4$ method. While added tellurite did not affect bacterial growth (Fig. 4A), the remaining tellurite in the supernatant dropped approximately to one-third after 3 h (Fig. 4B). Tellurite determinations were validated using, in parallel, the DDTC method (not shown).

Regarding the tellurite-resistant bacterium *A. caviae* ST, a 1:100 dilution of an overnight culture was inoculated into fresh LB medium and the OD_{600} was recorded at the indicated times. When the OD_{600} was ~0.4, the culture was amended with tellurite (400 μg/ml final concentration) (Fig. 5A, arrow) and the remaining tellurite in the supernatants was assessed as described above. Figure 5B shows that in 4 h ~27% of the toxic oxyanion was removed from the culture medium.

In summary and in comparison to the DDTC procedure, the $NaBH_4$ method described here allows more sensitive determination of the initial tellurite concentrations as well as the continuous uptake of the toxicant by tellurite-sensitive and tellurite-resistant microorganisms. This method should be of great help in future studies aimed at unveiling the tellurite reductase activity exhibited by some metabolic enzymes such as nitrate reductase (1), catalase (4), and the pyruvate dehydrogenase complex (5, 6). These studies are currently being carried out in our laboratory.

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