PERFORMANCE OF RADIOACTIVE MEASUREMENTS BY GAMMA SPECTROMETRY WITH SEMICONDUCTOR DETECTORS USING BIPHASIC SAMPLES

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ABSTRACT

An adaptation of the solid-liquid extraction technique has been developed to determine Hg-203 by gamma spectrometry with a semiconductor detector using biphasic samples, as a part of a study on the retention of mercury on the external membrane of bacterial cells. Three procedures have been tested to extract the radioactive isotope. One of them, consisting of a one-stage extraction using an optimized liquid volume, was seen to be preferable in this case. The three procedures might be useful to monitor the uptake of a contaminant (radioactive or not) by a solid phase, especially in small samples.

INTRODUCTION

A variety of techniques have been applied to the separation of toxic elements. Sorption of metals by microorganisms has proved useful in different types of samples (1). Living and dead cells are able to retain certain elements, like mercury, in a selective way (2). The monitoring of mercury in environmental and biological samples is of great concern because of the high toxicity of this metal. The use of a very sensitive technique like gamma spectrometry may be appropriate to determine mercury in very low concentrations.

Some series of experiments on the retention of mercury on the external membrane of bacterial cells were to be carried out using Hg-203 as a radiotracer (3). The size and shape of the samples are important factors to consider in gamma spectrometry with semiconductor detectors. The use of monophasic samples is usually considered an indispensable condition in this technique. However, it was difficult to obtain homogeneous samples with a specified shape from the small amount of bacteria used in the experiments. On the other hand, repeated separation of phases by solid-liquid extraction may lead to a decrease in accuracy. In these cases the performance of radioactive measurements in biphasic samples could be an option.

MATERIALS AND METHODS

A Canberra GR 2520 high purity Ge shielded detector was used for counting the 279.2 keV gamma rays of Hg-203 , in combination with a Canberra System 30 Plus multichannel analyzer. Detector efficiencies were calculated and radioactive measurements were optimized before gamma counting.

The Hg-203 radiotracer solution was in the form of mercury (II) chloride. The specific activity of the labeled solutions was 522.5 Bq/μg Hg. Nitric acid and other chemicals used in

this study were of the highest quality.

The bacterial cells used were Escherichia coli and Pseudomona putida. They were first cultivated in a solid medium TSA at suitable conditions. The bacteria were pelleted and lyophilized.

A 25 mL aqueous solution containing the radioactive mercury was equilibrated with 25 mg of the lyophilized bacteria for 30 minutes at room temperature at the working pH. The bacteria were separated from the supernatant by centrifugation at 12400 g, and then treated with a fixed volume of a 3.6 M nitric acid leaching solution. The amount of mercury was determined by gamma spectrometry in the biphasic sample obtained and in also in the supernatant. At least three measurements were made for each point.

Living cells, cultivated in a TSB medium, were also used. The density of the bacterial suspension was controlled by measuring the absorbance at 600 nm. Then the suspension was treated as in the lyophilized cell experiments.

RESULTS AND DISCUSSION

A problem was initially present because counting was made in a Hg-biomass pellet treated with a nitric acid solution and a part of the Hg could still be retained by the pellet. The optimization of radioactive measurements by the extraction process was performed in three ways.

One-stage extraction and use of an empirical factor

The following equation was used,

$$\Sigma C_t = \Sigma C_1 (E_p/E_1) + \Sigma C_p f \qquad [1]$$

where C_t are the total counts, C_1 and C_p are the counts from the supernatant and pellet, and E_p and E_1 are the detector efficiencies for the radiation from the pellet and supernatant, respectively. The efficiency varies because the volume of the biphasic sample was 4 mL, while that of the supernatant was 25 mL. The summatory means that counting was made in a number of different samples. An empirical statistical factor is introduced taking into account that the mercury content in the biomass pellet is not homogeneously distributed into the 4 mL volume. The values found for this factor, f, were 0.591 for E. coli and 0.517 for P. putida.

Two-stage extraction

The following set of equations was derived,

$$C_p = C_{12}(E_p/E_1) + xE_0 \qquad [2] \qquad x = (1-s)y \qquad [3]$$

$$y = C_{12}/E_1 s \qquad [4] \qquad C_{p2} = C_p(1-s) \qquad [5]$$

$$z = C_1/E_1 \qquad [6] \qquad P(\%) = [y/(z+y)]\,100 \qquad [7]$$

where C_{p2} are the counts in the second pellet sample (the second biphasic sample obtained after the separation of the 4 mL nitric acid solution and treating again the pellet with a 4 mL nitric acid solution, C_{12} is the counting in the supernatant from the first 4 mL volume after dilution to 25 mL, x is the

number of gamma rays emited in the time unit by the Hg present in the solid phase of the first biphasic sample, y and z have the same meaning as x in relation to the Hg content in the whole first biphasic sample and in the first supernatant, respectively, E_0 is the detector efficiency for samples situated at the bottom of the sample container, s is a separation factor meaning the fraction of Hg present in the biomass and extracted by the leaching acid solution going to the solution named "12", and P(%) is the percentage of the Hg initially retained by the pellet. The separation factor values were 0.92 for E. coli and 0.60 for P. putida.

One-stage extraction using an optimized volume

The usual one-stage extraction was employed combining it with volumes of solution varying from 4 to 100 mL in order to obtain the optimum volume. The equation used was

$$C_t = C_1(E_4/E_{25}) + C_p(E_4/E_v) \qquad [8]$$

where E_4, E_{25} and E_v are the detector efficiencies for samples with a volume of 4, 25 and v mL, respectively. We have found that the counts from the biphasic samples (corrected for the different efficiencies) decrease with the incresing volume of leaching acid. For volumes greater than 25 mL there is no variation, so the optimum volume is assumed to be 25 mL.

The standard errors of the mean values obtained using identical samples by the three extraction procedures, in the same order as described, were respectively 3.1%, 1.7% and 1.4% for E. coli, and 9.2%, 4.0% and 3.3% for P. putida. Using 4 mL of extractant liquid the results are only approximate. However, using 25 mL of leaching acid the Hg is completely released. In the case of P. putida the acid leaching is less effective, and hence the errors are greater.

CONCLUSIONS

Three procedures to measure gamma radiation from biphasic samples have been tested. One-stage extraction and use of an empirical statistical factor is a simple procedure needing less measurements, but it is less accurate. The two-stage extraction procedure improves the accuracy, but the operational complexity is greater. One-stage extraction with an optimized volume of liquid had the best accuracy and was preferable in this case, though more measurements are needed. The three procedures might be useful, according to the circumstances, especially in small biological or other types of samples, and particularly in cases in which the distribution of a contaminant (radioactive or not) between two different media should be monitored.

REFERENCES

1. Chin-Pin Huang, Chin-Pao Huang, A.L. Morehart, Wat. Res., 24, 433 (1990).
2. P.G. Mitchel, B. Green, J. Sneddon, Mikrochim. Acta, 1, 249 (1986).
3. A.J. Aller, J.M. Lumbreras, L.C. Robles, G.M. Fernández, 32, 511 (1995).