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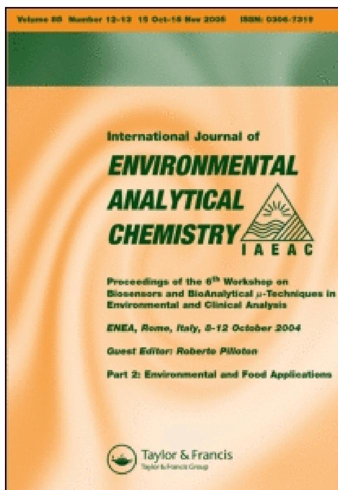

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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To cite this Article Pang, F. Y. , Ng, Y. L. , Phang, S. M. and Tong, S. L.(1993) 'Determination of Total Tin and Tributyltin in Biological Tissues', International Journal of Environmental Analytical Chemistry, 53: 1, 53 — 61
To link to this Article: DOI: 10.1080/03067319308045982
URL: http://dx.doi.org/10.1080/03067319308045982

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Intern. J. Environ. Anal. Chem., Vol. 53, pp. 53-61
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DETERMINATION OF TOTAL TIN AND TRIBUTYLTIN IN BIOLOGICAL TISSUES

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(Received, 17 July 1992; in final form, 5 February 1993)

Analytical procedures have been developed for the determinations of total tin and tributyltin in biological tissues using graphite furnace atomic absorption spectrometric (GFAAS) technique. For total tin determination, the acid digested sample solution of the tissues is allowed to pass through an anion exchanger column in the chloride form in order to remove the dissolved solids and residual lipids which interfere with the GFAAS measurements. Tin chloride is recovered in the eluate with 4M HNO_3. For the measurements of tributyltin, the acid-homogenised tissue solution is extracted directly with *n*-pentane. Dibutyl- and monobutyltin and inorganic tin are removed by back-wash with 0.8M NaOH. The extract is further cleaned up by passing through a C_{18} solid phase extraction (SPE) tube. Tributyltin recovered in a methanol-nitric acid mixture is determined by GFAAS using tungstate-treated graphite tube. The method developed has been applied for the analysis of bivalve samples and a detection limit of 0.4 ng g^{-1} as TBT-Sn has been achieved. The accuracy of the method has been checked using a certified fish tissue reference material (NIES-11). The levels of total tin and tributyltin obtained are in good agreement with the certified values.

KEY WORDS: Total tin, tributyltin, biological tissues, graphite furnace AAS, solid phase extraction.

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INTRODUCTION

Tributyltin (TBT) has been extensively used in marine antifoulants for many years, mainly as antifouling paint preparations for the coating of the hulls of ships, marine structures and in aquaculture cages. The ecotoxicological effects and bioaccumulation of TBT in coastal and marine fauna and flora, including commercially important bivalves such as cockles, oysters, clams etc. have been reported in various studies[1-3].

The assessment of the impacts arising from TBT pollution requires highly sensitive and specific analytical methods for the specific determinations of TBT in the water column as well as in the aquatic organisms. Numerous methods have been reported for the determination of TBT in marine tissues. Generally, these methods involve a separation or pre-concentration step by hydride generation, derivatisation—gas chromatography, high-performance liquid chromatography, or by chelation and liquid/liquid or liquid/solid extraction[4-9]. Subsequent detection is achieved by techniques such as flame and GFAAS,

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fluorimetry and flame photometry or mass spectrometry[10]. Among these analytical methods, the derivatisation—gas chromatographic-flame photometric detector (GC-FPD) technique requires careful sample preparation in achieving quantitative yield. The hydride generation—cryogenic preconcentration coupled with atomic absorption spectrophotometric or FPD detection also have been investigated extensively. It appears that although the cryogenic preconcentration could provide the necessary separation of the TBT species and high sensitivity, the inherent problems due to water condensation greatly limit its reproducibility and suitability for routine applications.

The present study is aimed at developing relatively simple analytical procedures for the determination of total tin and TBT in biological tissues which can be readily adopted for routine monitoring applications. The method has been designed to provide the necessary analytical sensitivity and specificity. The method developed also builds in sufficient sample pretreatment to ensure that reproducible and accurate detection by graphite furnace atomic absorption spectrometry can be achieved with minimum interference from sample residues.

MATERIALS AND METHODS

Apparatus

An Instrumentation Laboratory Model 251 atomic absorption spectrophotometer and a Perkin Elmer Model 2380 atomic absorption spectrophotometer were coupled to a Perkin-Elmer HGA-300 graphite furnace atomizer and used. Tin hollow cathode lamps employed were from Photron Pty. Ltd. (Sydney, Australia) and S & J Juniper & Co. (Essex, UK). The lamps were operated at a current of 15 mA and 10 mA, respectively, and with tin absorption wavelength at 235.5 nm as recommended by the manufacturers. Comparisons of sensitivity at this wavelength with those at 224.6 and 286.3 nm have been carried out. A deuterium continuum lamp was used for the non-atomic absorption correction.

Tungstate-impregnated pyrolytic graphite tubes were used in the measurements. The tubes were prepared by soaking prefired pyrolytic graphite tubes overnight in 5% (w/v) sodium tungstate solution. The impregnated tube was dried at 120° C for 2–3 hours before use and conditioned in the graphite tube assembly by applying the atomization programme adopted for tin measurement for five times.

The graphite furnace was operated using the following programme:

STEP	TEMPERATURE	RAMP	HOLD
Drying	120° C	10 s	30 s
Ashing	800° C	20 s	60 s
Atomizing	2600° C	1 s	4 s
Cleaning	2600° C	0 s	3 s

Argon was used as the purging gas and stop-flow was applied during the atomization step. Normally 10 µl or 20 µl aliquots of sample extracts were injected.

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Materials

All chemicals used were of analytical reagent grade. HPLC grade methanol (BDH '*Hipersolv*') and high purity UHQ water (Elgastat UHQ PS, Elga U.K.) were used in all preparations. Inorganic tin standard solution (1000 mg l^{-1}) was prepared from Merck '*Titrisol*' ampoule. TBT chloride in the liquid form and dibutyltin chloride in the salt form were purchased from Fluka Chemie AG (at least 97% purity) and stored at 4° C. The TBT chloride was used as it was without further purification.

Inorganic tin standard solutions (1 and 10 mg l^{-1}) were prepared by diluting from the 1000 ppm stock with 10% (v/v) HCl. Working standards at μg l^{-1} levels were made by further dilution in 4M HNO_3. A 100 mg l^{-1} TBT chloride stock was prepared in methanol. Working standards at μg l^{-1} levels were made by dilution in methanol-nitric acid (5% HNO_3) solution.

The C_{18} solid phase extraction tubes were purchased from Supelco Inc. as Supelclean LC-18, 40 μm monomerically bonded particles, 60 Å pores, 500 mg/3 ml. A certified fish tissue reference material (NIES-11) was obtained from the National Institute for Environmental Studies, Japan Environment Agency for checking the accuracy of the method for total tin and tributyltin. The reference was kept at -20° C upon receipt.

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Procedures

Bivalve samples Seven bivalve samples (six *Anadara granosa* and a *Paphia* sp.) and a green mussel (*Perna viridis*) were obtained from local market places in Kuala Lumpur and Petaling Jaya areas. Tissues were homogenised in a blender with the addition of 1:10 ratio of distilled water to assist blending if necessary. The homogenised tissues were stored in 100-ml wide mouth glass bottles and kept frozen in a freezer.

Total tin determination One gram of thawed, homogenized bivalve tissues was weighed into a 50-ml long-necked borosilicate digestion flask. For recovery studies, 100 μl of 10 mgl^{-1} inorganic tin and the same level of TBT were added prior to digestion. Concentrated HNO_3 (20 ml) was added and heating to boiling was carried out on a heating mantle for about 2 hours. The acid-digestate volume was evaporated until ca. 2 ml. After cooling, 10 ml of concentrated HCl was added and the acid-digestate solution was transferred quantitatively to a 25 ml volumetric flask and made up to volume with UHQ water. The NIES-11 reference sample (1.000 g) was treated following the same procedures.

Tributyltin determination Five grams of thawed, homogenized bivalve tissues were weighed and transferred quantitatively to a 250 ml borosilicate flat-bottomed round flask. For recovery studies, 100 μl of 1 mg l^{-1} TBT were added. Concentrated HCl (40 ml) was then added and the content was stirred with a magnetic bar for approximately 3 hours. Ten ml of UHQ water were added and stirring for another 10 minutes was carried out. The content was extracted with 100 ml of *n*-pentane. The *n*-pentane extract was then back-washed with 100 ml of 3% (w/v) NaOH solution. The *n*-pentane extract was evaporated to dryness at about 45° C and the residue was reconstituted with 10 ml of methanol-water (3:2). The NIES-11 reference sample (1.000 g) was subjected to similar treatments.

Sample clean-up For total tin analysis, the acid digested sample solution was allowed to pass through a strongly basic anion exchange resin (Dowex 2X8, 100–200 mesh). The column was prewashed with 4M HNO_3 and UHQ water and preconditioned with 50 ml of 2M HCl. Ten ml of the sample solution were loaded. This was followed by washing with 10 ml of 2M HCl. The absorbed inorganic tin was eluted with 4M HNO_3. Complete recovery of inorganic tin were collected in a total of 25 ml of the eluate. Tin concentration in the eluate was determined by injecting directly in the GFAAS.

The extracts for tributyltin determination were cleaned up using solid phase extraction (SPE) tubes prior to GFAAS measurements. The C_{18} SPE tube of 3-ml capacity was preconditioned with 2 ml of methanol followed by 2 ml of UHQ water. A positive pressure was used to effect a steady elution rate of 2 ml min^{-1}. The extracts in 10-ml methanol-water were allowed to pass through the tube. The column was then washed with 3 X 2 ml of methanol-water and finally eluted with 2 ml methanol-nitric acid. The eluate was collected in a glass vial and stored at 4° C. GFAAS measurements of the TBT concentration were performed by means of both standard calibration and standard addition methods.

RESULTS AND DISCUSSION

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Three spectral lines, *viz.* 224.6 nm, 235.5 nm and 286.3 nm of comparable sensitivity are available for atomic absorption measurement of tin. The relative sensitivities are dependent to a certain extent on the hollow cathode lamp characteristics and the atomization conditions. Results from the sensitivity measurements based on the calibration curve obtained for each of the spectral lines show that for both of the hollow cathode lamps employed, the sensitivity ratio was 0.5:1:0.8 for 224.6 nm:235.5 nm:286.3 nm lines. The noise levels of the 235.5 nm and 286.3 nm lines were comparable but significantly better than the 224.6 nm. The 235.5 nm line has hence been used for all the measurements in this study.

The graphite tubes used were impregnated with tungstate solution. The impregnated tungstate permits ashing at temperatures up to 800° C without significant loss of the analyte element, possibly due to the formation of stable Sn-W oxides[11]. Improvement in sensitivity by a factor of ten has been reported by Apte and Gardner[12]. A four-fold increase in sensitivity in tin has been recorded in the present study. The tungstate treated pyrolytic graphite tubes could be fired for 200–300 atomization cycles without significant loss in sensitivity.

Determination of total tin

For total tin determination, the common digestion using concentrated nitric acid is able to decompose the biological matrices completely except for refractory organic residues such as the higher lipids. The refractory organic carbon and the presence of significant amount of inorganic constituents in the solution can cause serious interference to the determination of total tin at very low level by the GFAAS technique.

Matrix modifiers such as ammonium dihydrogen phosphate have been recommended to overcome the problems introduced by the inorganic constituents. However, this was found

Table 1 Recovery of total tin and tributyltin from spiked samples.

Sample	*Spiked* Sn^{4+}, μg	*Spiked* *TBT-Sn*, μg	*Recovery** %
TOTAL TIN			
Anadara granosa #1	1.00	—	92
Anadara granosa #1	—	1.00	105
Anadara granosa #2	1.00	—	99
Paphia sp.	1.00	—	95
TRIBUTYLTIN			
Anadara granosa #1	—	0.100	97
Anadara granosa #2	—	0.100	92
Paphia sp.	—	0.100	98

*Triplicate means with variations in the range of 5–9%.

unsuitable for the present GFAAS system and very high non-atomic absorption was observed. Based on the atomic absorption and nonatomic absorption profiles obtained with GFAAS system, it was found impossible to determine Sn directly in the digested solution.

We have therefore tried to overcome the matrix interference from both organic and inorganic origin by a simple separation procedure. Based on the strong absorption of $SnCl_4^{-2}$ and $SnCl_6^{-2}$ on strongly basic anion exchange resin, interfering matrix species such as the salts of alkali and alkaline-earth elements and the refractory organic matter are easily separated after loading the sample and then rinsing the column with dilute HCl. The absorbed $SnCl_6^{-2}$ were quantitatively eluted with 4M HNO_3. Table 1 shows the recovery of spiked Sn^{4+} from digested tissues ranging from 92 to 99%. Quantitative recovery (105%) of tin added as TBT has also been obtained. Analysis of the certified fish tissue reference material (NIES-11) gave a total tin level of 2.6 ±0.1 μg g^{-1} from triplicate runs as compared to the certified value of 2.4 ±0.1 μg g^{-1}.

Table 2 shows the results of the total tin contents in a number of bivalves and green mussel samples. The total tin concentrations range between 1.0 to 3.2 μg g^{-1}. In comparison, McKie[4] reported 2.37 μg g^{-1} wet weight of total tin for the whole body of oyster (*Crassostrea gigas*).

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Table 2 Total tin and tributyltin contents in random bivalve samples.

Sample	*Common name*	*Total Tin* (μg g^{-1})	*TBT* (ng TBT-Sn g^{-1})
Anadara granosa #1	Cockles	3.2	1.0
Anadara granosa #2	Cockles	2.5	ND*
Anadara granosa #3	Cockles	1.0	2.0
Anadara granosa #4	Cockles	1.0	0.8
Anadara granosa #5	Cockles	2.8	—
Anadara granosa #6	Cockles	2.1	—
Perna viridis	Green Mussel	1.5	9.6
Paphia sp.	Bivalve	1.0	1.6

*ND = Nondetectable (<0.4 ng g^{-1} TBT-Sn)

Determination of tributyltin

Bivalve tissue was homogenised and dissolved in concentrated hydrochloric acid to facilitate the extraction of TBT into suitable organic solvents. *n*-Pentane has been chosen as the solvent for the extraction because of its low boiling point (36° C) and lesser susceptibility to emulsion formation[12]. The homogenised solution was extracted with two successive volumes of *n*-pentane of 100-ml each to ensure quantitative recoveries of TBT from the samples. The results from GFAAS determination of TBT extracted in the two successive fractions indicate that 93 ± 2% (n=8) of TBT were extracted in the first fraction and the second fraction contributes only a few percent. A single volume of 100 ml *n*-pentane has therefore been used for the extraction of TBT in later analyses.

Since the main aim was to achieve specific determination of TBT, the separated *n*-pentane layers were back-washed with 0.8M NaOH. This has been proven to exclude effectively the inorganic tin species as well as the mono- and dibutyltins from the organic layers[6]. Studies using spiked inorganic and di- and tributyltins indicate that the alkaline back-wash was effective in the removal of inorganic and dibutyltin and quantitative recovery of spiked TBT has been achieved (Table 1). Possible losses of TBT during evaporation of the organic extract were investigated and quantitative recoveries have been noted.

In the initial assessment, the evaporated residue of the NaOH-washed *n*-pentane extracts were taken up in 5 ml methanol/nitric acid mixture and TBT-Sn was then measured directly by injection into the GFAAS. Typical results of the GFAAS tin atomic absorption signals of the cockle tissue extracts with and without standard additions are shown in Figure 1. The

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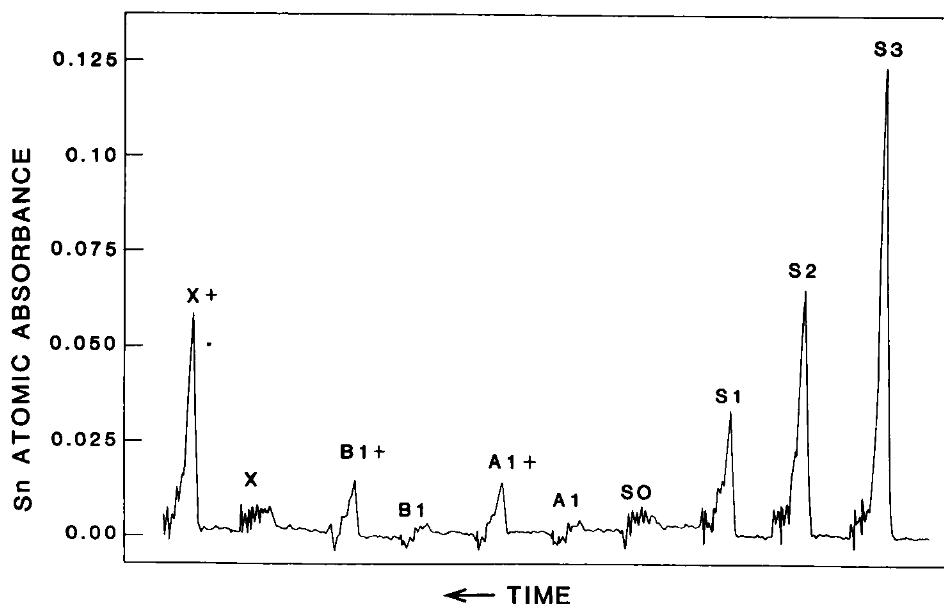

Figure 1 Recorder tracing of TBT-Sn atomic absorption signals of TBT standards (0, 20, 50 and 80 μg l^{-1} for S_0, S_1, S_2 and S_3 respectively) and cockle tissue extracts before (A1 & B1 without addition; A1+ & B1+ with addition of 50 μg l^{-1} each) and after passing through the SPE column (X without addition; X+ with addition of 50 μg l^{-1}).

tin atomic absorption of standard TBT spiked directly to the sample solution prior to the GFAAS injection was found to be grossly suppressed (A1+ & B1+ in Figure 1) even with the background correction. Loss of TBT by volatilization during the ashing step due to the presence of relatively high levels of oils and lipids co-extracted with the TBT has been attributed as the main cause of the signal suppression. The atomic absorption profiles also reveal severe matrix interference due to the refractory organics.

Recognising the nature of the interference problems, and in view of the simplicity and potential use of the solid phase extraction (SPE), an intermediate step of sample clean-up using C_{18} SPE has been introduced. In the SPE column separations, oil residues were noticeable in the methanol washings from the SPE tube. Also shown in Figure 1 are GFAAS tracings for the spiked and unspiked (X and X+) extracts of a cockle tissue sample after SPE clean-up. The atomic tin absorption profiles indicate a matrix interference-free TBT extract has been achieved. The SPE elution profile indicates that the spiked TBT was recovered quantitatively in the first fraction of the methanol-nitric acid eluate. Recovery of standard TBT spiked prior to the sample tissue treatment as shown in Table 1 ranges between 92 to 98%. Results in Table 1 support the manufacturer specified purity of the TBT chloride standard (97%) used in this study.

Calibration graphs based on peak height were constructed using TBT standards prepared in the same solvent conditions. However, standard additions have also been performed for most of the samples and minor matrix interference effects were corrected using the standard addition curves. The sensitivity obtained based on the slope of the calibration graph was 0.0014 $(\mu g\ l^{-1})^{-1}$, whereas the detection limit for TBT in tissues was estimated as 0.4 ng g^{-1} of sample. The sensitivity achieved using the tungstate treated graphite tube is approximately four times better than the non-treated ones.

Figure 2 shows a plot of the standard calibration in comparison with the standard addition curves for TBT for four different tissue samples. The slopes of some of the standard addition samples are significantly lower than the standard calibration curve. It is concluded that standard addition check is necessary to correct for any minor matrix interference to the atomic absorption measurements.

The procedures developed has been tested through the analysis of the NIES-11 certified fish tissue reference material. The average tributyltin chloride content of 1.4 ±0.1 $\mu g\ g^{-1}$ obtained in triplicate analysis is in good agreement with the certified value of 1.3 ±0.1 $\mu g\ g^{-1}$.

The TBT levels of a few local bivalves (*Anadara granosa* and *Paphia* sp.) and green mussel (*Perna viridis*) samples have been determined based on the standard addition method. The results are shown in Table 2. The contents of TBT-Sn found range from less than 0.4 ng g^{-1} to 9.6 ng g^{-1} wet weight. TBT-Sn contents of 0.18 $\mu g\ g^{-1}$ and 15–25 ng g^{-1} have been reported in oyster (*C. gigas*) in U.K.[4] and Canada[8], respectively.

CONCLUSIONS

Direct determination of total tin and tributyltin in digested and solvent extracted biological tissue samples, respectively, by graphite furnace atomic absorption spectrometric technique were not possible due to serious matrix interferences. Simple column clean-up methods

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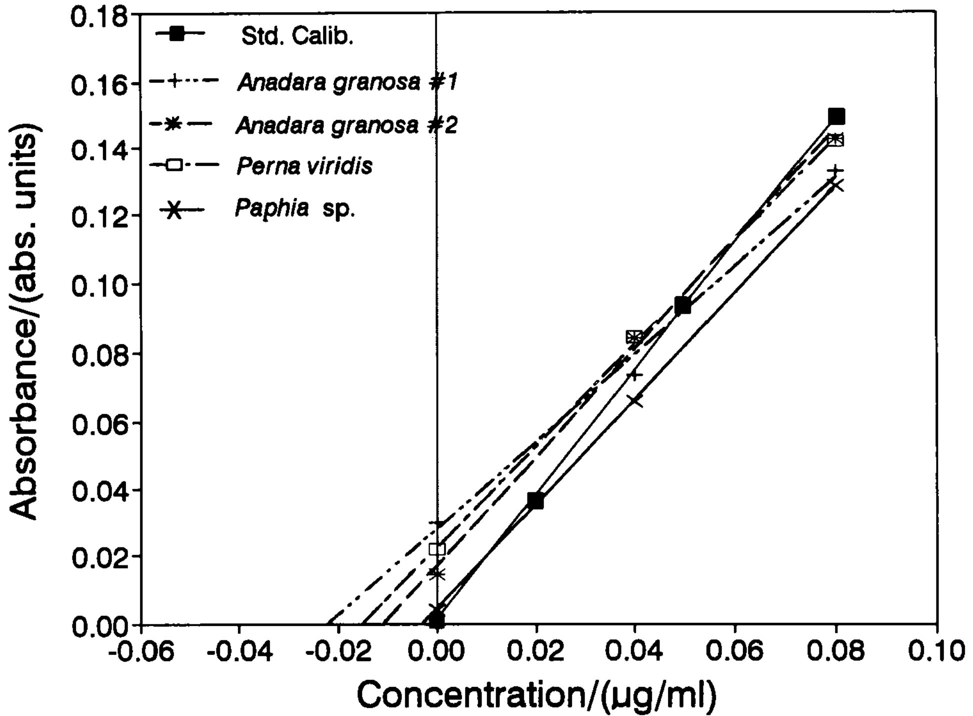

Figure 2 Comparison of the slopes of standard addition curves of cleaned-up sample solutions with the slope of standard calibration curve.

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using anion-exchanger for total tin and solid-phase extraction column for tributyltin have been developed. Quantitative recoveries and interference-free GFAAS determinations with enhanced sensitivity and reliability have been achieved.

Acknowledgements

This work was supported by a grant (R&D 4/07/04/56-01) from the Ministry of Science, Technology and the Environment of Malaysia and a short term grant (PJP 148/91; to F.Y.P.) from the University of Malaya. We are grateful to H. C. Lai for helpful advice and discussion.

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