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A novel application of nylon membranes for tributyltin determination in complex environmental samples by fluorescence spectroscopy and multivariate calibration

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ABSTRACT

The widely distributed pollutant tributyltin (TBT) was analyzed in different environmental samples (waters and sediments) combining preconcentration on a nylon membrane, excitation–emission fluorescence matrices directly measured over the membrane and second-order multivariate calibration. The latter was implemented using unfolded partial least-squares with residual bilinearization (U-PLS/RBL), a flexible algorithm achieving second-order advantage, even under severe spectral overlapping among sample components. Matrix-specific calibrations were required to overcome matrix effects, resulting in good analytical performance. TBT was determined in the concentration ranges 0.043–1.42 ng Sn mL^{-1} in water and 24–400 μg Sn kg^{-1} in sediments, with adequate detection limits in the range 0.03–0.15 ng Sn mL^{-1}.

1. Introduction

Tributyltin (TBT) is presumably one the most toxic agents introduced into the environment by human activities. Due to its extensive use in numerous fields, large amounts of TBT have been introduced into various ecosystems, especially in the aquatic environment, where the disturbing impact on the life cycle of aquatic organisms constituted the first alarm [1,2]. TBT has also shown considerable toxicity towards living organisms, including aquatic organisms, mammals and humans, presenting high environmental persistence and ability to transfer along the trophic chains [3,4]. The use of organotin compounds in anti-fouling paints was completely banned in 2008 by the International Convention on the Control of Harmful Anti-fouling Systems on Ships of the International Maritime Organization, and the European Union has included TBT in its list of priority compounds in water [5]. However, high TBT levels are still reported in environmental samples [6,7] and in aquatic systems. TBT bonds to suspended material and sediments, where it can remain and be released for up to 30 years [3]. According to this information, the monitoring of TBT levels in environmental samples is mandatory.

Analytical methodologies previously employed to assess the TBT levels in environmental samples involve hyphenated techniques requiring a combination of extraction, separation and detection steps [8,9]. For preconcentration, previous proposals include liquid–liquid [10] and solid phase extraction [11], as well as solid phase [12] and liquid phase microextraction [13]. For separation, gas chromatography (GC) coupled to selective detector systems has usually been employed [14]. Although the analytical performance of these methodologies is widely recognized, they are complex, time-consuming, require substantial experimental work and skilled analysts and are difficult to implement for routine analysis.

Modern multi-way calibration methods, on the other hand, are an attractive alternative to complex sample analysis, even when the response signals are less selective than chromatography [15,16]. In particular, second-order multivariate calibration allows one to determine analytes in the presence of uncalibrated constituents, a property known as the 'second-order advantage' [17,18]. It is known that by increasing the order of the multivariate approach, method sensitivity can be improved [19]. Common algorithms employed to analyze first-order data are partial least-squares (PLS) [20], while for second-order data, the most employed ones are parallel factor analysis (PARAFAC) [21], multivariate curve resolution-alternating least-squares (MCR–ALS) [22,23] and some latent-structured methods, such as unfolded partial least-squares (U-PLS) and multiway PLS, both combined with

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residual bilinearization (RBL) [24,25]. This chemometric strategy to fluorescence data has already been applied to quantify triphenyltin [26] and tributyltin [27] in natural waters, after derivatization with flavonoids in aqueous media. However, these latter methods require an additional preconcentration step to reach the environmental triorganotin levels, and they have only been applied to the analysis of natural waters.

In the present report, we propose a new approach for the quantitation of TBT in natural waters and also in more complex samples, such as marine sediments, resorting to preconcentration with a nylon membrane, followed by direct surface measurement of fluorescence signals for the fluorescent TBT complex. The use of nylon as a medium to extract, preconcentrate and enhance luminescence signals is already well-established in the literature [28–30]. The present report is an additional example of the successful combination of solid surface fluorescence matrix spectroscopy and multivariate calibration.

2. Experimental

2.1. Apparatus

A Varian Cary-Eclipse luminescence spectrometer (Mulgrave, Australia) equipped with a xenon flash lamp was used to obtain the emission spectra and the excitation–emission fluorescence matrices (EEFMs). The EEFMs were recorded on the nylon surfaces exciting the samples in the range of 360–520 nm (each 5 nm) and obtaining the corresponding emission spectra in the range of 470–700 nm (each 2 nm), resulting in a data matrix of size 116 × 33 for each sample. The widths of the excitation and emission slits were 5 nm. The emission spectra were obtained by exciting the samples to 430 nm and obtaining the emission spectra in the range of 470–700 nm (each 2 nm). The spectral data were saved in ASCII format and transferred to a computer for subsequent manipulation. EEFM data pre-treatment included removal of Rayleigh and Raman signals [31].

For fluorescence measurements on a solid phase, a KS13 stainless steel syringe holder purchased from Advantec, MFS, Inc. (Dublin, CA) and nylon membrane filters (13 mm × 0.2 μm, Nyflato™) purchased from PALL Life Sciences (Cortland, USA) were used. Similar spectroscopic behaviours were observed for all tested nylon membranes. The latter did not require any conditioning and were used as-received.

All glassware was rinsed with deionized water, decontaminated overnight in a 20% (v/v) nitric acid solution (Merck, Darmstadt, Germany) and then rinsed again with deionized water.

2.2. Reagents and standards

High-quality water (18 MΩ) obtained from a Barnstead Easypure II (Thermo Scientific, Dubuque, MA USA) was used to prepare the solutions. The tributyltin chloride standard (TBT, 96%) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions of this reagent (1000 mg L^{-1} of Sn) were prepared in methanol (HPLC grade, LiChrosolv®, Merck S.A.) and stored at −20 °C in the dark. Working standards were prepared weekly for aqueous solutions of 10 mg L^{-1} Sn (storage at 4 °C), and daily for solutions containing nominally 10–100 μg L^{-1} of Sn in water. A 4.2×10^{-3} M methanol solution of morin (Sigma-Aldrich, Munich, Germany) was prepared weekly and kept at 4 °C protected from light.

2.3. Sorption and fluorescence spectroscopy measurements on nylon surfaces

For the sorption process, a nylon membrane was spotted with 10 μL of 6.25×10^{-5} M methanol solution of morin, aided with a micropipette and dried for a few seconds on a plate at about 50 °C. The disk was then loaded into the stainless steel holder syringe and connected to a 3 mL plastic syringe. To concentrate the TBT–morin complex onto a restricted area of the nylon surface and thus increase the sensitivity of the method, a poly-tetrafluoroethylene (PTFE) ring was fitted over the membrane before the interaction between TBT and morin. In this way, a nylon surface with a final diameter of 5 mm was exposed to the flowing solution. An adequate volume of each aqueous TBT solution was forced to pass through the membrane in approximately 1 min. The excess of liquid was removed by twice forcing 3 mL of air through the disk using the same syringe. The membrane was then removed from the stainless steel holder and completely dried on a plate at about 50 °C. For EEFM measurements, the disk was kept on a home-made holder, and placed into the spectrofluorimeter in such a way that the angle formed between the excitation and emission beams was 90°, with an incident angle of 45°. Additional instrumental conditions were described above. A new membrane was used for each analysis.

2.4. Calibration set

A set of synthetic solutions with analyte concentrations between 0 and 1 μg (Sn) L^{-1} was used for calibration. To verify the prediction ability of the multivariate method, the predicted concentrations were compared with the nominal values using the classical approaches including root mean squared error of cross-validation (RMSECV) and prediction (RMSEP), and relative error of prediction (% REP).

For the analysis of natural water and sediment extract samples, a calibration set was prepared in the corresponding background matrices, due to a significant matrix effect with respect to calibration in deionized water. Previously, these samples were analyzed with a liquid–liquid extraction-gas chromatographic method with pulsed flame photometric detection (LLE-GC-PFPD) [32], and no detectable levels of organotin compounds were found (<7.0 ng g^{-1}). The quality of the multivariate calibration model was evaluated in these matrices using RMSECV, RMSEP and % REP errors.

2.5. Real samples

The aqueous samples considered in this study were drinking, lagoon and seawater samples collected from different sites of the Valparaiso Province (Region de Valparaiso, Chile). The drinking water samples were collected from the drinking water system of Valparaiso City. The lagoon samples were collected from the "Laguna de la Luz" located in Curauma Village. Finally, the seawater sample was collected from the Yacht Club in Viña del Mar City. After collection, all samples were filtered using a cellulose membrane (0.45 μm) and stocked at 4 °C until analysis.

Surface sediment samples from the coast of Iquique (Region de Tarapaca, north of Chile) were collected by specialized staff from the Chilean Army. Iquique is a northern Chilean city, in which dry-docking and harbor/commercial activities are currently carried out. The collected samples were freeze-dried, sieved at 63 μm and stored in plastic bottles at −20 °C until analysis. These samples were labeled E-1 (Punta Cavancha) and E-2 (Caleta Cavancha). TBT concentrations were also found to be below the detection limit according to GC-PFPD measurements. For the extraction of the analyte from sediment samples, two common approaches were used: glacial acetic acid, previously validated in ref. [33], and tartaric acid with 20% methanol [34]. Briefly, 1.0 g of marine sediment was placed in 30 mL polycarbonate centrifuge tubes and treated with 20 mL of either glacial acetic acid or tartaric acid solution (0.5 M) containing 20% methanol. The suspension was shaken at 400 rpm for 12 h in an elliptic mechanical shaker, centrifuged and immediately analyzed.

For the analysis of water samples, aliquots of 7.0 mL were pH adjusted to 5.0 with NaOH and diluted with deionized water until to 10.00 mL. For sediment extracts, 0.5 mL of sample was pH adjusted and diluted as described above for the water samples.

2.6. Theory

For first-order data, such as fluorescence emission spectra, the PLS algorithm has been widely used in analytical problems and its theory has been explained in several tutorials [20,35].

The theory of PARAFAC is also well-known [21]. For second-order analysis based on EEFM data processing, the latter algorithm is the first choice, because of the fact that its intrinsic model is based on the properties of the fluorescence signals [36]. Briefly, a set of EEFMs is arranged into a three-way mathematical object showing a property known as trilinearity. This property means that: (1) the signal for a given sample component depends on three parameters which are independent of each other: concentration, excitation profile and emission profile; and (2) the instrumental profiles are unique and do not depend on the sample. PARAFAC often achieves unique decomposition of the three-way data array, a fact that makes it the most employed algorithm for second-order EEFM analysis. However, in the presence of a high degree of overlapping of either excitation or emission profiles for two or more sample components, unique decompositions leading to successful analyte quantitation may be difficult [37]. Under the latter circumstances, a more flexible, latent-structure method such as U-PLS/RBL may be useful, as described below.

In the U-PLS method, the original second-order data are unfolded into vectors before PLS is applied, as has been described by Wold et al. [38]. Coupling to RBL is mandatory in order to achieve the second-order advantage. When unexpected constituents occur in the unknown sample $\mathbf{X}_u$, the PLS scores ($\mathbf{t}_u$) obtained are unsuitable for analyte prediction and the residuals of the test sample signal (s_p), contained in the matrix $\mathbf{E}_p$ (see below), will be abnormally large in comparison with the typical instrumental noise level. This can be represented by the equation:

$$s_p = \frac{\|\mathrm{vec}(\mathbf{E}_p)\|}{(JK-A)^{1/2}} = \frac{\|\mathrm{vec}(\mathbf{X}_u - \mathbf{P}\mathbf{t}_u)\|}{(JK-A)^{1/2}} \quad (1)$$

in which $\|\cdot\|$ indicates the Euclidean norm, $\mathbf{P}$ is the matrix of loadings, vec $(\cdot)$ implies the vectorization command, J and K are the number of instrumental channels in each data mode, and A corresponds to the number of PLS latent variables. To handle the presence of unexpected constituents, residual bilinearization resorts to the principal component analysis (PCA) of the contribution from the unexpected components [39], by minimizing the residuals $\|\mathbf{e}_u\|$ computed using a Gauss–Newton procedure. The latter fits the sample data to the sum of the relevant contributions [40]. For a single unexpected component, the expression is:

$$\mathrm{vec}(\mathbf{X}_u) = \mathbf{P}\mathbf{t}_u + \mathrm{vec}\left[g_{unx}\mathbf{b}_{unx}(\mathbf{c}_{unx})^T\right] + \mathbf{e}_u \quad (2)$$

where $\mathbf{b}_{unx}$ and $\mathbf{c}_{unx}$ are the left and right eigenvectors of $\mathbf{E}_p$ and g_{unx} is a scaling factor. The standard deviation (s_{RBL}) of the residuals in Eq. (2) can be used as a measure of the goodness of fit (GOF) for the RBL procedure. According to Bortolato et al. [41], s_{RBL} is given by Eq. (3)

$$s_{RBL} = \|\mathbf{e}_{RBL}\| / \left[(J-N_{unx})(K-N_{unx})-A\right]^{1/2} \quad (3)$$

where N_{unx} is the number of unexpected components. The latter number is usually estimated by inspecting the behaviour of s_{RBL} with increasing N_{unx}. The value of s_{RBL} is assumed to stabilize at the instrumental noise level when the correct value of N_{unx} is reached.

2.7. Software

All calculations were carried out using MATLAB 7.8 routines (The MathWorks Inc., 2009). PLS, PARAFAC and U-PLS/RBL were applied through a MATLAB graphical user interfaces [42,43] which are available on the Web at www.iquir-conicet.gov.ar/descargas/mvc2.rar.

3. Results and discussion

3.1. Solid-phase fluorescence strategy

The reaction between morin and organotins yields a fluorescent complex which previously allowed the spectrofluorimetric determination of these compounds [27,44]. The stoichiometry of these complexes is 1:1 [45] and appears to involve the 3-hydroxyl-4-keto moiety of the morin structure, because it offers higher delocalization of both the oxygen electrons and the π-electrons [46].

In the present report, and with the purpose of increasing the sensitivity of the method, a strategy involving a TBT solid-phase extraction and the direct measurement of the TBT–morin complex fluorescence in the solid surface is proposed.

In the first stage, several solid membranes such as polycarbonate, nylon, cellulose nitrate and vinylidene-fluoride (PVDF) were evaluated. For this purpose, morin and TBT–morin complex were loaded into two independent membranes, and the fluorescent emission of the complex was measured by subtracting the spectra obtained in the latter two membranes. Only nylon produced a significant fluorescent spectrum for the TBT complex, and thus this membrane was selected as support.

It was established that a stable complex between TBT and morin is formed at pH 5.0 [27,44], and thus this condition was selected for subsequent experiences. On the other hand, since preliminary experiments adding salts or organic solvents (i.e., methanol, acetonitrile) to the aqueous TBT solution led to a significant decrease of the fluorescent signal, these reagents were avoided.

Two loading procedures were evaluated. In Method 1, the fluorescent TBT–morin complex is firstly formed in aqueous solution, and then an aliquot is passed through the membrane and the complex is retained. In Method 2, on the other hand, a few microlitres of morin solution are adsorbed in nylon, and then the TBT solution is passed through the disk, generating the fluorescent product directly on the nylon membrane. The latter alternative has been previously reported for the development of a fluorimetric nylon probe for Hg(II) ion using univariate calibration [47]. The loaded membranes obtained with both protocols are presented in Fig. 1. Clearly, each approach retains different quantities of the involved chemical species on the membrane. As expected, no intense fluorescence is observed for the nylon membrane in the studied spectral region (Fig. 1A), although weak light scattering signals are detected. Following the first approach, the obtained EEFM for an analytic standard (Fig. 1B) present a significant intensity, similar to that obtained in aqueous solution [27]. However, poor reproducibility and significant non-linearity in the signal–concentration relationship were observed for the calibration curves obtained with this approach. These results could be ascribed to the presence of inner-filter effects produced by a high free morin concentration retained on the membrane, and/or to severe overlapping between the spectra for morin and TBT–morin complex. Therefore, the second loading strategy was adopted, even when some spectral artifacts, produced for the membrane, could still be observed (Fig. 1E and F).

A possible explanation for the morin retention mechanism on the nylon surface is the formation of hydrogen bonds between nylon C═O and NH groups on one hand, and the phenolic and keto groups of morin on the other. Similar sorption mechanisms have been previously reported for biological molecules on Nylon 66 [48]. It is conceivable that free groups of morin could interact with TBT, producing the fluorescent product which is detected on the solid surface. No measurable leaching of morin in the eluted aqueous solution was observed, indicating that the adsorption of morin is efficient enough to avoid significant loss when the aqueous TBT solution is filtered through the membrane. A new disk with fresh reactive surface is used in each measurement, and therefore the sensing capability of the probe remains unaltered. Indeed, the reproducibility of the obtained fluorescence spectra indicates that the adsorption mechanism in nylon provides an appropriate strategy

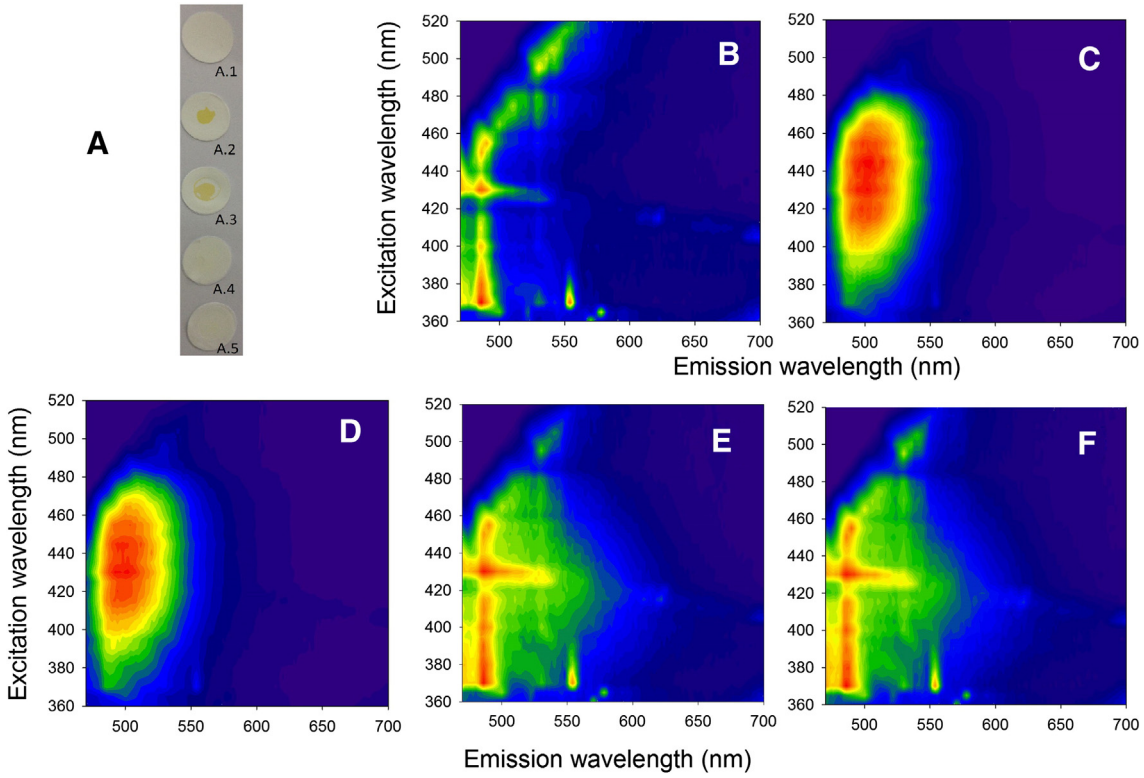

Fig. 1. (A) Different membranes obtained in optimal conditions containing: (1) no reagents, (2) reagent blank loaded using Method 1, (3) analytic standard loaded using Method 1, (4) reagent blank loaded using Method 2 and (5) analytic standard loaded using Method 2. Contour plot obtained in the latter five cases are shown in B, C, D, E and F, respectively.

with a minimum experimental effort and a very short experimental time (about 3 min per sample).

The selection of the volume and concentration of morin solution to be spotted over the nylon membrane was performed in the range of 5–20 μL and 6.25×10^{-4}–6.25×10^{-6} M, respectively. The results showed that a volume of 10 μL of 6.25×10^{-5} M morin solution was the most favorable one to deposit the reagent over the nylon surface.

The suitable equivalents to be deposited on the nylon surface were determined passing the same TBT solution through nylon membranes treated with increasing amounts of morin. Significant fluorescence intensity for TBT–morin complex is observed at TBT–morin ratio higher than 1:37. Clearly, this ratio is higher than the expected 1:1 stoichiometry for the TBT–morin complex. However, in accordance with our experience and previous results, an excess of morin is mandatory for TBT fluorescent measurements [27]. In the actual case, a fraction of morin previously deposited on the membrane could be released from the solid support when the sample is passed through the membrane decreasing the complexant equivalent for TBT complex formation. Thus, the protocol we followed (see above) guarantees an adequate TBT–morin ratio in the investigated concentration range. In relation to the volume of TBT solution filtered through the nylon membrane, it is well-known that in solid-phase retention methods, sensitivity can be improved by applying larger sample volumes [49]. However, when nylon membranes are used as an extractive support, large sample volumes (e.g. >50 mL) may lead to clogging problems, and the analysis time may become impractical [28]. Volumes from 1.0 to 5.0 mL of TBT solution were tested, and a value of 2.0 mL was shown to be appropriate for observing an adequate signal without involving long experimental times.

3.2. Quantitative analysis in synthetic samples

On the basis of the optimal conditions confirmed above, the spectrofluorimetric determination of TBT in nylon membrane was carried out. Several multivariate approaches could be considered for this purpose. In this study, we compared the analytical performance of the second-order algorithms PARAFAC and U-PLS/RBL. The application of PARAFAC to second-order rendered poor recovery results, probably because of the severe overlapping of fluorescent spectra of morin and TBT–morin complex. Therefore, only the U-PLS/RBL results will be discussed in the following sections.

The analytical parameters obtained with U-PLS/RBL for the calibration and validation set of samples are presented in Table 1. As expected,

Table 1
Statistical results[a] for TBT in deionized water by using U-PLS.

Algorithm	U-PLS
A[b]	4
RMSEC (ng mL^{-1})[c]	0.01
RMSEP (ng mL^{-1})[d]	0.09
REP (%)[e]	18.2
γ^{-1} (ng mL^{-1})[f]	325
LOD (ng mL^{-1})[g]	0.03

[a]Values obtained when 2 mL of sample solution are extracted on a nylon membrane of 5 mm exposed diameter size. Final TBT concentrations up to 1.0 ng mL^{-1} were included in the known linear range and no attempts were made to establish the upper concentration of the linear range since the goal was to detect low concentrations of TBT. [b]A, latent variables determinated by leave-one-out cross-validation. [c]RMSEC, root-mean-square error of calibration and [d]RMSEP, root-mean-square error of prediction were calculated in accordance with $RMSE = [(1/I)\sum_{1}^{I}(c_{nominal} - c_{predicted})^2]^{1/2}$ where I is the number of prediction samples and c_{nominal} and $c_{\mathrm{predicted}}$ are the actual and predicted concentrations, respectively. [e]Relative error of prediction, $REP = 100 * RMSEP/\bar{c}$ where $\bar{c}$ is the mean calibration concentration. [f]γ^{-1}, inverse of the analytical sensitivity. [g]LOD, limit of detection calculated according to ref. [50].

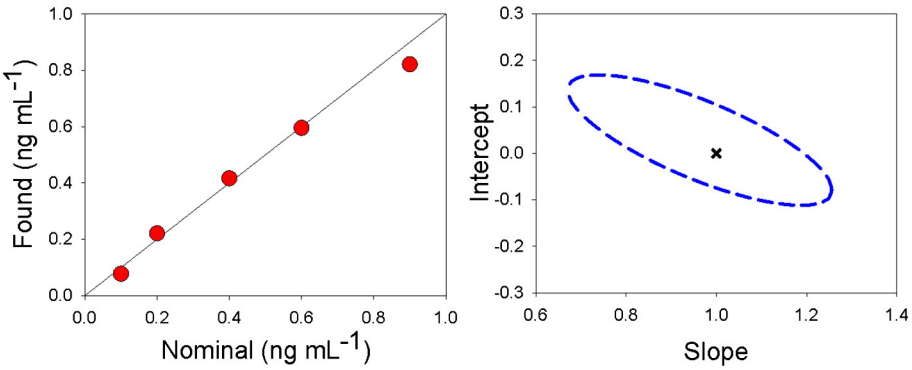

Fig. 2. Plot for U-PLS/RBL predicted concentrations of TBT as a function of the nominal values for validation set (A). (B) Elliptical joint regions (at 95% confidence level) for slope and intercept of the regression for U-PLS/RBL (dashed line) samples. Cross marks the theoretical (intercept: 0, slope: 1) point.

the number of latent variables required for U-PLS are higher than the expected chemical components, probably due to modelization of the spectral response observed for nylon membrane. Nevertheless, a significant improvement can be observed for the figures of merit (sensitivity, LOD) in comparison to direct measurement in solution as previously reported.

For prediction quality, the root mean squares error of calibration (RMSEC) and prediction (RMSEP) and relative error of prediction (%REP) were evaluated and presented in Table 1. These parameters are commonly used to validate a multivariate model and they represent the differences between the real and predicted concentrations for calibration and validation set. The prediction error (0.09 ng ml^{-1}) and relative error (18.2%) allowed us to assess the reliability of the proposed method for TBT determination. Besides, a good correlation between nominal and predicted concentrations was found for both models in accordance with Fig. 2A.

With the purpose of assessing the accuracy of the predicted concentrations, the elliptical joint confidence region (EJCR) test was performed [50]. The EJCR test consists of plotting, in the slope–intercept plane, the region of mutual confidence (usually at a 95% confidence level) of the slope and intercept for the regression of predicted vs. nominal concentrations. The region has an elliptical shape, and the test consists of checking whether the theoretically expected values of slope = 1 and intercept = 0 are included within the ellipse. When the ideal point is included within the EJCR, this indicates the accuracy of the used methodology. Fig. 2B shows that the EJCR obtained U-PLS results, demonstrating that there was no evidence of bias within the 95% confidence level.

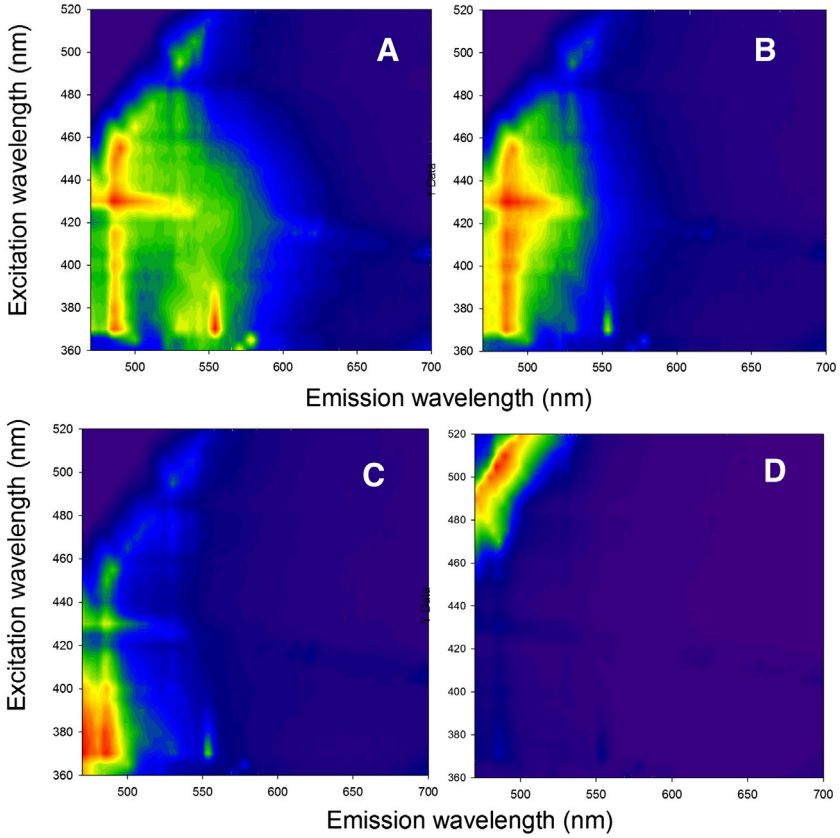

Fig. 3. Excitation–emission matrices obtained for (A) analytic standard, (B) lagoon, (C) seawater and (D) sediment samples under optimal conditions.

Table 2
Statistical results for TBT in spiked water and sediment samples using U-PLS/RBL[a].

Water samples	Lagoon	Seawater	Tap water
A[b]	4	4	2
RMSECV[c]	0.11	0.14	0.08
RMSEP (ng mL^{-1})[d]	0.05	0.02	0.03
REP (%)[e]	12	6	7
γ^{-1} (ng mL^{-1})[f]	210	180	187
LOD (ng mL^{-1})[g]	0.03	0.08	0.08
Sediment samples	E-1	E-2	
A[b]	1	3	
RMSECV (ng mL^{-1})[c]	0.09	0.04	
RMSEP (ng mL^{-1})[d]	0.06	0.03	
REP (%)[e]	13	8	
γ^{-1} (ng mL^{-1})[f]	630	458	
LOD (ng mL^{-1})[g]	0.15–0.17	0.06–0.08	
LOD (ng kg^{-1})	40–68	24–32	

[a]Values obtained when 2.0 mL of sample solution are extracted on a nylon membrane of 5 mm exposed diameter size. Final TBT concentrations up to 1.0 ng mL^{-1} were included in the known linear range and no attempts were made to establish the upper concentration of the linear range since the goal was to detect low concentrations of TBT.
[b]A, latent variables determined by leave-one-out cross-validation.
[c]RMSECV, root-mean-square error of cross-validation.
[d]RMSEP, root-mean-square error of prediction.
[e]REP, relative error of prediction.
[f]γ^{-1}, inverse of the analytical sensitivity.
[g]LOD, limit of detection calculated according to ref. [50].

3.3. Quantitative multivariate analysis of the environmental samples

Taking into account that environmental matrices may contain interfering constituents and lower detection limits are mandatory to reach environmental TBT levels, a second-order chemometric analysis was considered more adequate and evaluated. Some preliminary studies showed that PARAFAC rendered unsatisfactory results and thus this algorithm was not considered for the analysis of real samples. On the other hand, although there was severe spectral overlapping between free morin and the TBT complex, U-PLS/RBL was able to distinguish between these signals, rendering excellent results (see below). In relation with interferences, the fluorescent response observed for common degradation products of tributyltin, such as dibutyltin and monobutyltin, after sorption on the nylon membrane was negligible. It can be attributed to the low fluorescent response shown for these butyltins (pH 5.0), as previously reported [44]. Finally, the applicability of the proposed method was tested by adding analytes to real samples, such as water and sediment samples found to be TBT-free, and performing recovery studies.

3.3.1. Natural waters

Initially, under optimal working conditions, EEFMs were recorded for calibration samples, some of which are shown in Fig. 3. In the case of the water samples, seawater produced a different fluorescence landscape in comparison with an analytic standard, as can be observed in Fig. 3C and A, respectively. In this sample, it is likely that the presence of metallic cations could be responsible of the observed changes, by forming stable complexes with morin, in accordance with previous reports [27,51]. The fluorescent response for dissolved organic material was not observed, probably due to the fact that this material produces a detectable luminescent response at lower wavelengths [31]. Table 2 presents the results obtained after modelling the second-order data with U-PLS/RBL. Interestingly, the number of components estimated by cross-validation is similar in these samples with respect to analyte standards measured in deionized water. Obviously, no RBL components are necessary due to matrix modeling during calibration.

Fig. 4A shows the prediction results corresponding to the application of U-PLS/RBL to the real water samples. The good results suggest that the method can overcome the problem of the presence of unexpected interferents from environmental background matrices. In the present case, the ideal (1,0) point lies inside the EJCR surface (Fig. 4C), suggesting that U-PLS/RBL is appropriate for resolving the system under investigation. The corresponding statistical results shown in Table 2 are also indicative of high-quality predictions.

As regards the limits of detection (LODs), it is important to consider the low concentration levels of TBT admitted by governmental agencies in environmental samples. Clearly, the LOD estimated for the proposed methodology does not reach the Environmental Quality Standards (EQS) established for the European Union Directive 2013/39/EU (0.0015 ng mL^{-1}). However, the present methodology is applicable for the common levels reported recently in waters from different countries where the TBT ban is not adopted or is non-existent [3,52–54].

As can be appreciated in Table 2, the low LODs attained are very favorable, taking into account the complexity of some of the evaluated systems, such as lagoon and seawaters, and the simplicity of the experimental determination. It is necessary to point out that these limits have been calculated according to the novel IUPAC-consistent estimator suggested by Allegrini and Olivieri for PLS calibration [55]. This approach combines mathematical and analytical criteria, leading to a new LOD estimator which adopts the form of a detection interval, as shown in Table 2.

3.3.2. Sediment samples

The retention of TBT–morin complex on nylon could be affected by the nature of the aqueous media in which the complex is immersed, e.g. the extracting solution media. In the literature, several alternatives have been proposed for organotin extraction from sediment samples [33,34]. In this work, the effect of different volumes of acid extract (between 0.5 and 2.0 mL) passed through the membrane was studied on the self-predicted concentration values for the calibration set. The best results were obtained for 0.5 mL for both extracting media. Higher extracting solution contents could lead to a decrease in the free-TBT fraction available to react with morin, due to complexation with tartaric and acetic acid. Likewise, lower prediction errors were obtained for

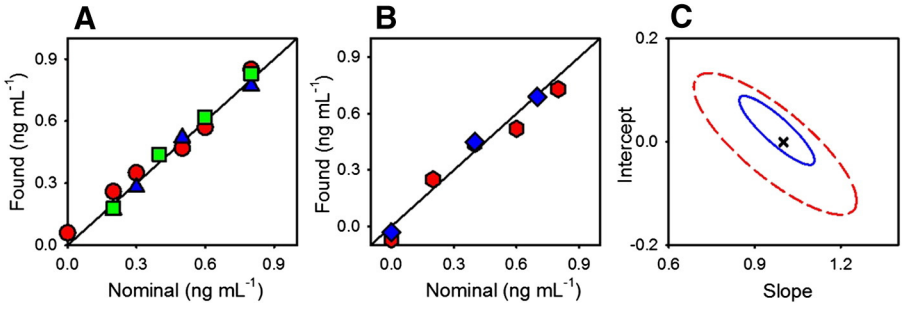

Fig. 4. Plot of U-PLS/RBL predicted concentrations of TBT as a function of the nominal values in lagoon (circles), seawater (triangles) and tap water (squares) (A), in two different sediment (diamonds and hexagons) (B). (C) Elliptical joint regions (at 95% confidence level) for slope and intercept of the regression for waters (solid line) and for sediments (dashed line). Cross marks the theoretical (intercept: 0, slope: 1) point.

Table 3
Comparative figures of merits for analytical methodologies commonly used to determine tributyltin in environmental samples.

Method	Compounds	Sample	LOD	RSD	Recovery	Analysis time	Ref.
HSSE-TD-GC-MS	MMT, DMT, MBT, DBT, MOcT, DOcT	Natural water; leachates from plastic bottles	1.7–7.0 ng L^{-1}	<10%	–	135 min	[55]
Ethylation-DLLME-GC-PFPD	TBT, DBT, MBT, TPhT, DPhT, TPhT	Seawater	0.2–1.0 ng L^{-1}	2.3%–5.9%	82.5%–104.7%	24 min	[13]
Ethylation-LLE-GC-PFPD	TBT, DBT, MBT, TPhT, DPhT, TPhT	Sediments, wastewater	0.2–1.0 ng Sn L^{-1}	1%–8%	91%–103%	45 min	[10]
HS-SPME-GC-PFPD	MMT, DMT, TMT, MBT, DBT, TBT, MPhT, DPhT, TPhT, MOcT, DOcT	Human urine	0.5–4.9 ng Sn L^{-1}	2%–7%	87%–104%	70 min	[56]
Propylation-LLE-GC-MS	TBT, DBT, MBT, TPhT, DPhT, MPhT	Coastal sediments	6.6–13 μg g^{-1}	11%–14%	–	45 min	[57]
MAE-Ethylation-GC-MS	TBT, DBT, MBT, TPhT, DPhT, MPhT, MOcT, DOcT, TOcT	Sewage sludge	9–42 ng Sn g^{-1}	2%–8%	–	80 min	[58]
Ethylation-LLE-GC-MS (tartaric)	TBT, DBT, MBT	Surface sediment samples	42–52 ng Sn L^{-1}	7%–8%	–	30 min	[34]
Ethylation-LLE-GC-ICP-MS	TBT, DBT, MBT	Freshwater sediment and mussel samples	3.4–5.6 ng kg^{-1}	<1%	89%–107%	20 min	[59]
Fluorescence-multivariate calibration	TBT	Natural waters	7.0 ng L^{-1}	<14%	85%–120%	40 min	[27]
Fluorescence-multivariate calibration	TBT	Sediments, natural waters	24–40 ng kg^{-1}	6%–13%	85%–113%	5 min	This work
			0.03–0.15 ng mL^{-1}				

acetic acid, which can be attributed to higher extraction yields in comparison with methanolic tartaric acid, as previously reported [34]. A typical excitation EEFM obtained for a sediment extract is presented in Fig. 3D.

As in real water systems, the recovery results obtained for sediment samples (Fig. 4B), the corresponding EJCR test (Fig. 4C) and the remaining statistical results (Table 2) were also very satisfactory.

In Table 3, the figures of merit for methods recently reported for the determination of TBT are summarized and compared with the proposed method. As can be seen, the application time for the proposed methodology is significantly lower than other protocols, requiring only 5 min in comparison to 20 or 30 min for the fastest alternatives currently available. On the other hand, the present detection limits are higher than the most sensitive techniques, such as gas chromatography (GC) coupled to mass spectrometry or inductively coupled plasma-mass spectrometry detection. However, they are comparable to routine analysis methods based on liquid–liquid or solid phase extraction followed by GC with common photometric detection (FPD or PFPD).

4. Conclusions

Immobilization of morin on a nylon membrane in combination with second-order multivariate calibration provides an environmentally friendly and sensitive fluorescence probe for TBT determination in natural samples. Nylon is demonstrated to be a suitable support for these purposes, allowing the complex formation reaction to occur on its surface. Excitation–emission fluorescence matrices for the TBT–morin complex were directly measured in the solid surface and processed by U-PLS/RBL, allowing the successful determination of the organotin compound in the presence of matrix interferences. The use of nylon saves organic reagents (morin and the solvent where it is dissolved) in relation with the solution system, and improves the sensitivity of the method. The application of a chemometric tool makes it unnecessary to apply additional clean-up steps for the removal of interfering compounds, saving experimental time and operator efforts. Through the simple and inexpensive proposed methodology, TBT was successfully determined at trace levels in environmental samples, with the advantage that good results were obtained with a minimal sample treatment, and without using organic solvents.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.chemolab.2015.09.005.

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