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Characterization of newfound natural luminescent properties of melamine, and development and validation of a method of high performance liquid chromatography with fluorescence detection for its determination in kitchen plastic ware

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ABSTRACT

Experimental studies of the natural photoluminescence of melamine in aqueous solutions showed that its fluorescence intensity (at 250/365 nm) was appropriated for analytical purposes. The exploitation of such melamine property provided the basis of development of a new, simple, precise and accurate method based on high performance liquid chromatography with fluorescence detection (HPLC–Fluo) to determine melamine in kitchen plastic ware following aqueous extraction using a microwave oven. Optimization of analytical parameters such as solvent composition, pH and extraction conditions led to limits of detection and quantification of melamine of 0.0081 and 0.027 $\mu g\ mL^{-1}$, respectively, with a linear range up to 10 $\mu g\ mL^{-1}$. Sample extracts fortified with melamine at three concentration levels produced an average recovery of 98 ± 6%, which was in agreement with the results achieved with a reference HPLC–UV method. Different samples of kitchen plastic ware analyzed by the developed and optimized method showed melamine concentrations in the aqueous extract up to 17 $\mu g\ mL^{-1}$, which corresponded to 86.0 $mg\ kg^{-1}$ in these utensils. The results obtained indicate that the use of kitchen plastic ware made of melamine can contaminate food with this compound after heating in a microwave oven.

1. Introduction

Melamine (1,3,5-triazine-2,4,6-triamine) is produced from urea under heat and pressure. Its reaction with formaldehyde produces a melamine–formaldehyde resin (MF) [1], which is a very durable thermo set plastic, commonly known and commercialized by the generic name of "melamine". MF is widely used for the manufacture of a variety of kitchen plastic ware and utensils consumed worldwide, especially because of their low prices and surface properties in terms of hardness, chemical stability and transparency, which allow brilliant visual appearance of the surface and good performance during the use [2].

Despite these desirable characteristics, it is not possible to ensure that the MF resin used for the production of these apparatus is homogeneous throughout its length because the complexity and reversibility of the condensation reaction between melamine and formaldehyde that occurs in water at a certain pH, temperature and solids content [3]. Undoubtedly, one of the most worrying effects of this reaction is that residual melamine and/or formaldehyde monomers may remain in the plastic ware after manufacturing, and consequently released to food during the lifetime of these products [4]. Previous studies showed that leaching of both compounds from the MF into food was strongly affected by heating and acidity [5–8]. The Federal Institute for Risk Assessment (BfR) showed that the Tolerable Daily Intake (TDI) for melamine (0.2 $mg\ kg^{-1}$), established by The European Food Safety Authority (EFSA), was clearly exceeded when foods were heated in kitchen utensils made of melamine resins using a microwave oven at temperatures up to 70 °C [9].

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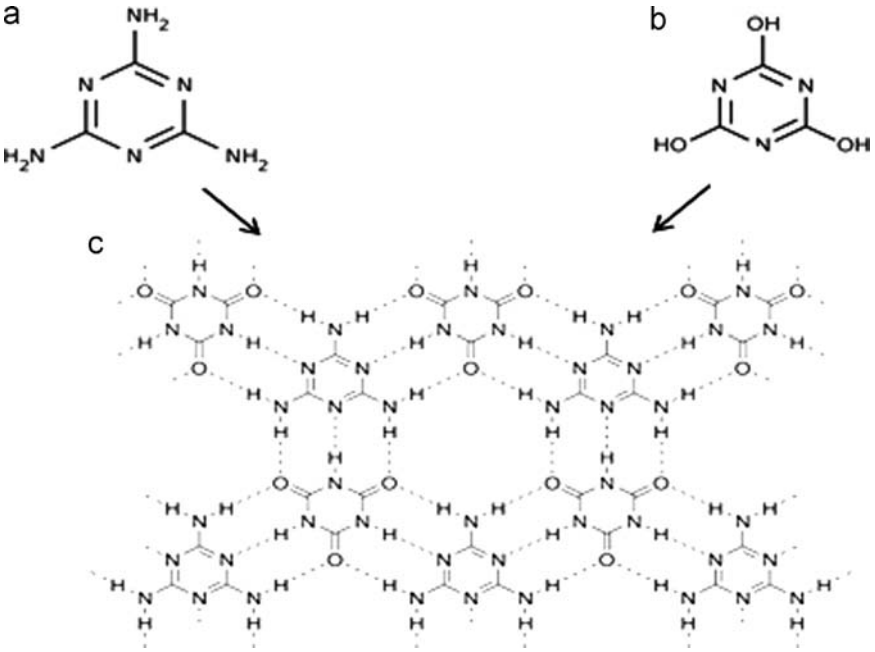

Fig. 1. Chemical structures of (a) melamine; (b) cyanuric acid and (c) melamine cyanurate complex.

The exposure to melamine may be particularly dangerous to human health because it may form stones in the urinary system what might lead to acute renal failure by obstruction. Consequently, a variety of renal toxic effects such as nephrolithiasis, chronic kidney inflammation and bladder carcinoma [10] can result, notably when melamine forms a stable melamine–cyanurate complex with cyanuric acid (Fig. 1).

A lamentable example of the toxic effects of melamine occurred in China in 2008, where more than 294.000 children ingested an infant formula adulterated with melamine, and at least six of them died [11]. A World Health Organization (WHO) expert meeting report [12] described the toxicological aspects and health effects of melamine and cyanuric acid and recommended TDIs of 0.2 and 1.5 mg per kg of body, respectively. Two very recent reviews about the advances in the risk assessment of melamine and cyanuric acid discussed the impacts of these compounds, particularly of melamine and its analogues, in animal feed [13] and infant formula [14].

Owing to the relevance of melamine, several selective and screening methods were recently proposed for its quantitative determination [15,16]. HPLC deserves a detached role among them, because it may be used for the determination of melamine in various matrices, with high accuracy, sensibility and good reproducibility. Most of the HPLC methods used for the quantitative determination of melamine employed UV [17–20], MS [21] or MS/MS [22,23] detection.

HPLC–UV methods commonly used for melamine determination in food products provide sufficient sensitivity in accordance to international regulations. However, the fact that many organic compounds absorb at 240 nm may led to erroneous quantification or over-estimative of melamine if the method is improperly validated in terms of sample preparation and chromatographic conditions [24]. On the other hand, high selectivity and reliability are achieved using HPLC–MS/MS, but the use of this technique increases considerably the analysis cost.

A number of new methods based on photoluminescence were recently proposed for the determination of melamine: complexation with a fluorescent sensor (cucurbit-7-uryl, for example) [25], electroluminescence enhancement of $Ru(bpy)_3^{2+}$ [26], fluorescence enhancement of gold nanoparticles [27,28], reaction with neutral red [29], and through the use of the Mannich reaction [30]. However, none of these studies investigated the direct measurement of the natural fluorescence of melamine.

The fluorescence of a compound depends of its molecular structure. A relatively rigid and planar structure, with specific symmetry and an electron-rich π system, capable to allow $\pi^* \rightarrow \pi$ transitions (π-antibonding to π-bonding orbitals), is favorable to the phenomenon of fluorescence. The surrounding environment, such as the solution composition and pH, also has effect on the luminescent characteristics of the molecule, affecting not only the rate of the luminescent and nonradiative transition processes (intersystem crossing, internal conversion, vibrational relaxation), but also the nature and relative energy of the low energy excited state [31].

Therefore, the effect of pH in the fluorescence of solutions of molecules containing basic or acid functional groups is important because there may be significant differences in the electron distribution of protonated and non-protonated luminescent molecules. An aspect of interest is the production of species of higher fluorescence quantum efficiency due to protonation, deprotonation or acid/basic hydrolysis. Protonation or dissociation can alter the nature and rates of nonradiative processes, competing with the photoluminescence phenomenon, and thereby can affect the quantum emission yields [31]. All conditions described above are found in the structure of melamine, and at least hypothetically, they may favor the fluorescence of this compound. Moreover, the amino groups of present in the molecule would tend to amplify its fluorescence because of the increase of the rate constants of radiative decay [31].

The aim of this paper is to present an experimental study of the photoluminescent behavior of melamine solutions and show how this property was applied for the development of a new, simple, precise and accurate method of determination of melamine in kitchen plastic ware by HPLC with fluorescence detection (HPLC–Fluo). The optimization of experimental conditions, such as solvent composition and pH, ensured a high fluorescence intensity of the analyte.

As far as we are aware, this is the first study showing the natural fluorescence of melamine and its analytical application through a HPLC–Fluorescence method. Besides sensitivity, one of

the main advantages of this new method is the use of the fluorescence of aqueous solutions of melamine, without any derivatization step.

2. Material and methods

2.1. Reagents and solutions

All chemical reagents were of analytical grade or better. Ultrapure water (resistivity of 18.2 MΩ cm^{-1}) was prepared through a Simplicity system (Millipore, USA) following reverse osmosis (Rios D3, Millipore, USA). Melamine ($\geq$ 99%, Sigma-Aldrich, USA) was used to prepare standard solutions. Ammonium chloride (HPLC grade, Merck, Brazil) and acetonitrile (HPLC grade, Tedia, Brazil) were used to prepare the mobile phase of HPLC–Fluo analysis. TFA (Sigma-Aldrich, USA) and methanol (HPLC grade, J.T.Baker) were employed to prepare the mobile phase of the HPLC–UV separation. Sodium hydroxide and acetic, boric, phosphoric and hydrochloric acids of analytical grade, were obtained from Merck (Brazil).

Standard stock solutions of melamine (100 mg L^{-1}) were prepared by dissolving an appropriate mass weighed in an analytical balance (GR-202, AND, Japan) in ultrapure water. The aqueous solution was kept in an ultrasonic bath (Unique, Brazil) for few minutes until complete dissolution of the solid. The standard solutions of melamine (0 to 10 μg mL^{-1}) employed to obtain the analytical curves were prepared by dilution of appropriate aliquots of the stock solution.

Solutions of the Britton-Robinson buffer (0.04 mol L^{-1}) were employed in the pH study. They were prepared by mixing aqueous solutions of acetic acid, boric acid and phosphoric acid in the same proportions. The pH of the buffer solutions (from 2 to 12) were adjusted by addition of a concentrated solution of sodium hydroxide, with the aid of a pH-meter (Digimed, model DM-22, SP, Brazil).

2.2. Spectrofluorimetric measurements

Fluorescence measurements employed a luminescence spectrophotometer (Cary Eclipse model, Varian, USA). Fluorescence spectra were acquired using the SW Eclipse® Bio Pack V 1.1 (XP WIN2000) software. Quartz cuvettes (1 cm optical path length), 20 nm spectral band pass and 1200 nm s^{-1} scan velocity were used. The intensity of fluorescence of melamine solutions was measured at its wavelengths maxima of excitation (250 nm) and emission (365 nm), after optimization of the selected parameters (pH and solution composition).

2.3. HPLC–Fluo system and chromatographic conditions

Chromatographic analysis was performed using high-performance liquid chromatography with fluorescence detection (HPLC–Fluo). The chromatographic system consisted of a vacuum degasser, a quaternary pump, an automated injector, a column oven and a fluorescence detector, and was controlled by an Agilent ChemStation. A Zorbax SB-C18 (150 × 4.6 mm, 5 μm) column connected to a Zorbax ODS (12.5 × 4.6 mm, 5 μm) pre-column was used for chromatographic analysis. The mobile phase consisted of an aqueous solution of ammonium chloride (10 mmol L^{-1}) and acetonitrile (98:2% v/v) that were degassed in an ultrasonic bath and filtered using a Millipore filtration system and 0.45 μm membranes (Agilent, USA) prior to use. The mobile phase flow rate was 0.8 mL min^{-1}. An injection volume of 20 μL was used for standards and samples solutions and the column temperature was 25 °C. The wavelengths of maxima excitation (250 nm) and emission (365 nm) were used to allow the best detection sensitivity in the determination of melamine.

2.4. HPLC–UV system and chromatographic conditions

In order to evaluate the performance of the HPLC–Fluo method, samples were simultaneously analyzed by HPLC–UV using a previous published method [18], which was considered a reference method, with few adaptations to the laboratory conditions. The chromatographic system consisted of a vacuum degasser, a binary pump, an automated injector, a column oven and a UV-DAD detector, and was controlled by an Agilent ChemStation. A Zorbax SB-C18 (250 × 4.6 mm; 5 μm) column connected to a pre-column with the same characteristics was used for chromatographic analysis. The mobile phase consisted of a mixture of an aqueous solution of TFA 0.1% (pH 2.4) and methanol (90:10%, v/v) that was degassed in an ultrasonic bath and filtered using a Millipore filtration system and 0.45 μm membranes (Agilent, USA) prior to use. Isocratic conditions and a flow-rate of 1.0 mL min^{-1} were used. An injection volume of 10 μL was used for standards and samples solutions and the column temperature was 25 °C. The detection was carried out at 240 nm and the retention time of melamine was 3.0 min.

2.5. Samples description and treatment

The studied samples were obtained from 10 different brands of kitchen plastic ware made of melamine–formaldehyde resin purchased in stores of the cities of Rio de Janeiro and Niterói, in Rio de Janeiro State, Brazil. The samples were broken into small pieces in order to increase their homogeneity and contact surface of the samples during extraction. Melamine extractions from kitchen utensils were performed by heating 5 g of the broken samples in 20 mL of ultrapure water, in a suitable covered glass flask, for 3 min in a domestic microwave oven (ME185, Electrolux, Brazil). This extraction condition was also chosen in order to simulate a possible use of this kind of plastic ware. After cooling to room temperature, the extracts were quantitatively transferred to 25 mL volumetric flasks. The samples intended for HPLC–Fluo analysis were reconstituted using ultrapure water, whereas for HPLC–UV, extracts were diluted using a mixture of aqueous methanol (50%, v/v). Aliquots of the extracts were filtered through syringe filters (0.22 μm, 25 mm, Millipore, USA), and transferred into glass vials for HPLC–Fluo analysis or quartz cuvettes for spectrofluorimetric measurements. Samples were analyzed immediately after extraction. The extraction kinetics was studied using both analytical techniques (HPLC–Fluo and spectrofluorimetry).

3. Results and discussion

3.1. Study of photoluminescent behavior of melamine solutions

The first step of the studies of melamine photoluminescence was the choice of the solvent system, considering both the analyte solubility and the signal intensity. Common laboratory solvents such as ethanol, acetonitrile and water were evaluated. Among them, ultrapure water was chosen due to the good solubility of melamine, but especially because of the intense fluorescence of this compound in water. Moreover, water represents an environmentally friendly solvent.

The excitation and emission spectra of an aqueous solution of melamine (1 μg mL^{-1}) showed an intense maximum of fluorescence emission at 365 nm after excitation at 250 nm (Fig. 2). A $\pi \rightarrow \pi^*$ transition, characteristic of monoaromatic rings, is possibly the main responsible of the excitation of melamine in aqueous

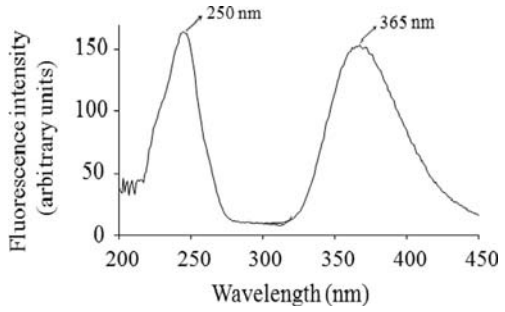

Fig. 2. Excitation and emission fluorescence spectra of an aqueous solution of melamine (1 μg mL^{-1}). Instrumental conditions: 20 nm spectral bandpass; 1200 nm s^{-1} scan velocity.

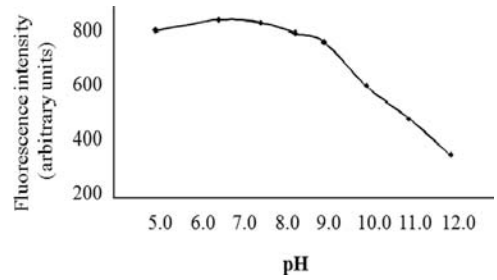

Fig. 3. Influence of pH on the intensity of fluorescence emission at 365 nm of melamine solutions (10 μg mL^{-1}) prepared in Britton-Robinson buffer. Instrumental conditions: 20 nm spectral bandpass and 1200 nm s^{-1} scan velocity.

solutions. These results were in very good agreement with our initial hypothesis of the fluorescent behavior of melamine solutions.

The subsequent step of this investigation was a preliminary study of the effect of pH upon the behavior of the fluorescent signal of aqueous solutions of melamine in strongly acidic or basic media (containing different concentrations of HCl or NaOH, respectively). The results obtained showed that melamine precipitated in acidic pH values (<4.5) and presented decreasing signals with increasing concentrations of NaOH (pH >12). Therefore, a specific study was made to better evaluate the influence of the pH of the analyte solution upon the fluorescence signal. With this purpose, melamine solutions were buffered with the Britton-Robinson buffer at pH values between 5 and 12. The melamine solutions showed intense and nearly constant fluorescent signals for pH values ranging from 5.0 to 7.5, leading to robust measurements of fluorescence. The fluorescent signal sharply decreased between pH 8.0 to 12, showing that the fluorescence intensity of melamine is strongly affected by pH (Fig. 3).

3.2. Determination of melamine in plastic ware by spectrofluorimetry

Owing to the demonstrated fluorescence of melamine in aqueous solutions, the first quantitative approach consisted in the evaluation of its concentration in plastic ware by direct spectrofluorimetry. With this purpose, aliquots of 5 g of the samples were extracted as described above (Section 2.5). The fluorescence spectra obtained in this part of the study showed a small shift of the wavelength of maximum emission of sample extracts when compared to those of the standard solutions of melamine, clearly indicating matrix interference (Fig. 4). This interference can be a result of other substances such as plasticizers and coloring agents used in manufacturing of melamine plastic ware that were co-extracted. Therefore, spectrofluorimetry was discarded as a method for quantitative determination of melamine

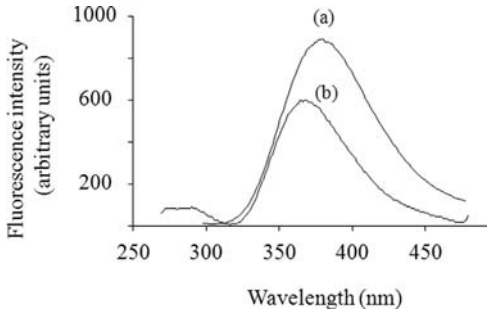

Fig. 4. Emission fluorescence spectra of (a) melamine standard solution (10 μg mL^{-1}) and (b) solution of non-spiked sample extract, obtained after of microwave oven heating of a kitchen plastic made of melamine–formaldehyde resin during 3 min. Instrumental conditions: 20 nm spectral bandpass and 1200 nm s^{-1} scan velocity.

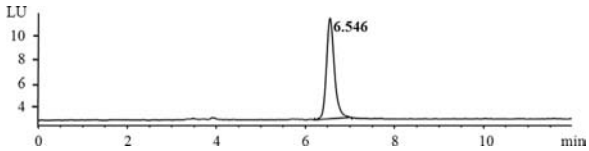

Fig. 5. Typical chromatogram of an aqueous standard of melamine (2.5 μg mL^{-1}) obtained in the optimized HPLC–Fluo conditions. See Section 2.3 for further details.

in aqueous extracts of plastic ware obtained after microwave oven heating. Consequently, the use of a separation method, based on HPLC–Fluo, was necessary for the continuity of the study.

3.3. Conditions of the HPLC–Fluo separations

The chromatographic method initially focused on the investigation of the factors, such as mobile phase composition and pH, which directly affect the retention of melamine in the C18 column. The mobile phase composition was adjusted to obtain an adequate retention time for melamine in order to avoid the elution of any possible interference elution. An aqueous solution of ammonium chloride (10 mmol L^{-1}) led to a symmetric peak of melamine and the effect of the addition of various proportions of acetonitrile to the mobile phase was tested. The results obtained showed that the retention time of the melamine decreased with increasing percentage of acetonitrile. The best solvent proportion between NH_4Cl 10 mmol L^{-1} and acetonitrile was 98:2% v/v, as described above (Section 2.3). The final pH of this solution was 5.5, which represents an intermediate value in the robust range of pH found for the fluorescence intensity of melamine, as discussed in Section 3.1 (Fig. 3). The retention time for melamine was 6.5 min (Fig. 5), without any coelution or interference.

3.4. Evaluation of the extraction kinetics

After establishing the separation and detection conditions, the extraction kinetics was evaluated in order to allow the validation of the analytical method. The influence of the microwave heating time on melamine extraction was studied using different samples. The microwave heating for 30 s was able to extract 5 g of kitchen utensils samples using water (20 mL), leading to measurable fluorescence signals or chromatographic peak areas. However, an extraction time of 3 min was employed because it was suitable for the simulation of a real situation of heating a beverage or food in a microwave oven, and the chromatographic area tended to a constant value (Fig. 6). The average temperature of the aqueous extract at the end of the extraction period was always beyond 100 °C.

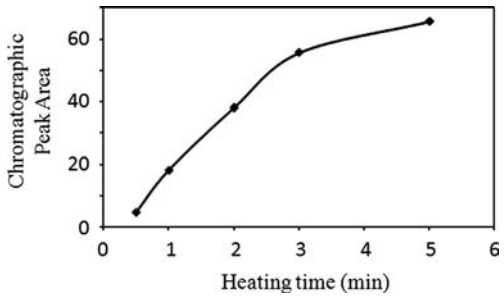

Fig. 6. Kinetics of aqueous extraction of kitchen plastic ware made of melamine–formaldehyde resin. Measurements made at the wavelength of maximum emission (365 nm), using 10 nm spectral bandpass and 1200 nm s^{-1} scan velocity.

Table 1
Analytical figures of merit of the developed HPLC–Fluo method.

Parameters	Results
Regression line[a]	Peak area = 44.33* (concentration of melamine)–0.619
Coefficient of determination (r^2)	0.9998
Concentration range	0.05 to 10 μg mL^{-1}
LOD[b]	0.0081 μg mL^{-1} (0.041 mg kg^{-1})
LOQ[b]	0.027 μg mL^{-1} (0.14 mg kg^{-1})
Repeatability[c]	0.8%
Intermediary precision[c]	1%
Robustness	pH range from 5.0 to 7.5

[a] Average of 3 curves.
[b] LOD and LOQ calculated according to the IUPAC criteria.

3.5. Validation of the performance of the HPLC–Fluo method

The performance of the HPLC–Fluo method was established through a validation protocol that followed the criteria of analytical methods validation established by the Brazilian National Institute of Metrology, Quality and Technology [32], which is harmonized with international regulations. The validation parameters studied were: sensitivity (linearity/linear range), limits of detection and quantification, repeatability and intermediate precision, robustness, recovery and selectivity. The analytical parameters of merit were obtained using the optimized HPLC–Fluo conditions used for the determination of melamine in kitchen plastic ware.

Three analytical curves were obtained by the least squares method using the chromatographic peak areas measure after injection of aqueous standards containing melamine (0.05 to 10 μg mL^{-1}). A representative analytical curve equation (confidence limit of 95%) was described by peak area = 44.33 × (concentration of melamine) − 0.619, with a coefficient of determination (r^2) of 0.9998. The absence of outliers at each concentration level was demonstrated by Jacknife residue test and the homoscedasticity of the data was confirmed by Levene test [33].

The limit of detection (LOD) and the limit of quantification (LOQ) were estimated according to the IUPAC criteria, respectively as $3s_b/m$ and $10s_b/m$, where s_b was the signal to noise ratio and m was the slope of the regression line. The signal to noise ratio was estimated by the standard deviation of peak area obtained after 10 subsequent injections of the less concentrated standard (0.05 μg mL^{-1}). The LOD and LOQ obtained were 0.0081 μg mL^{-1} (0.041 mg kg^{-1}) and 0.027 μg mL^{-1} (0.14 mg kg^{-1}), respectively.

The method repeatability was estimated using the relative standard deviation (RSD(%)) obtained after ten consecutive measurements of three standard solutions of melamine (0.5, 5.0, and 10.0 μg mL^{-1}), under the optimized HPLC–Fluo conditions. The RSD(%) obtained was lower than 0.8% for the three levels studied.

An intermediary precision of 1% was calculated based on the analysis of variance (ANOVA) of the data obtained by changing the analyst that performed the measurements. Each analyst prepared a set of independent melamine standards (0.5, 5.0, and 10.0 μg mL^{-1}) and obtained ten consecutive replicate measurements of the three standards (as described for the repeatability study) under the optimized HPLC–Fluo conditions.

The robustness of the method was evaluated by taking into account the influence of the mobile phase pH in the retention time and area of the melamine peaks. A robust condition, with no significant variation of peak area and retention time, was found for pH ranging from 5.0 to 7.5, thus confirming the results shown in Fig. 3.

Table 1 summarizes of the analytical parameters of merit of the HPLC–Fluo method described above.

Method trueness was evaluated through recovery studies of spiked samples, applying the extraction procedure and HPLC–Fluo method described above. The results obtained were compared with those obtained by simultaneous analysis of sample extracts using a previous published HPLC–UV method [18].

Three different samples spiked with known amounts of melamine at three different levels (0.5, 5.0 and 8.5 μg mL^{-1}) allowed recovery evaluation. Spiked and non-spiked samples were submitted to the extraction procedure (Section 2.5) and HPLC analysis. Recovery tests were performed on three consecutive days. The mean recovery obtained using HPLC–Fluo was calculated and compared to those obtained using the reference HPLC–UV method. Recoveries (%) were calculated dividing the difference of the measured concentrations of spiked and non-spiked samples by the spiked concentration and expressing this ratio in a percentage basis. The concentrations were determined by direct interpolation in analytical curves. The recoveries obtained by HPLC–Fluo were 98 ± 6% (mean ± standard deviation) showing the good accuracy of this method of melamine determination in plastic ware. This value showed a good accordance with the recovery found using the HPLC–UV method (100 ± 1%). The comparison of the recoveries obtained by both methods showed that they were statistically similar at a 95% confidence level.

The sensitivity of the HPLC–Fluo method is the main advantage comparatively to the HPLC–UV method. This finding can be illustrated by the LOQ obtained by HPLC–Fluo (0.027 μg mL^{-1}), that is almost an order of magnitude lower than the one obtained by HPLC–UV (0.2 μg mL^{-1}) [18]. This fact is reflected in the possibility of measuring lower concentrations of melamine (0.05 to 10 μg mL^{-1}) by HPLC–Fluo, when compared to those measured by HPLC–UV (1.0 to 80 μg mL^{-1}). These results evidence the large sensitivity of the HPLC–Fluo method, which allows the measurement and assessment of low concentrations of melamine that cannot be detected by HPLC–UV.

Furthermore, the overall volume of organic solvent (sample dilution and analysis) used in the HPLC–Fluo method is smaller than that used in the HPLC–UV method, making the proposed method cheaper and environmentally friendly.

3.6. Determination of melamine in kitchen plastic ware

The samples analyzed were of different origins and manufacturers and this fact was not taken into account in this study. The authenticity of most samples (MF resin) was checked by their infrared spectra (data not shown). (original position modified).

The identification of the chromatographic peaks of melamine in sample extracts was confirmed by the fluorescence emission spectra obtained online after excitation at 250 nm, simultaneously to the quantitative analysis. No wavelength shifts or new maxima

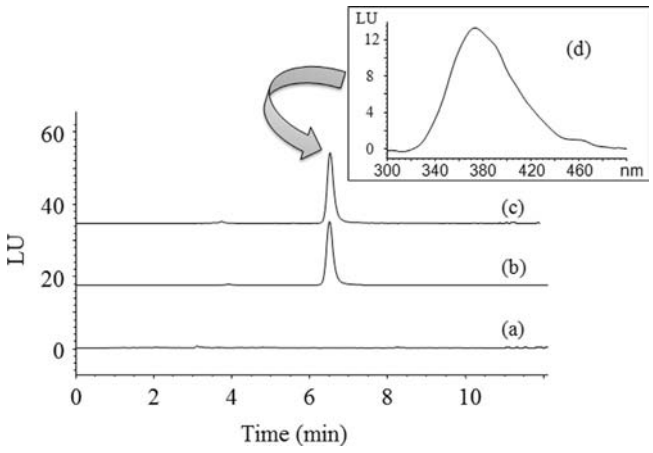

Fig. 7. Typical chromatograms of (a) ultrapure water; (b) aqueous standard of melamine (5.0 μg mL^{-1}); (c) solution of sample extract spiked with melamine (5.0 μg mL^{-1}). A fluorescence spectrum of a sample extracted, simultaneously monitored by fluorescence detection HPLC system (250/365 nm) is shown on (d).

Table 2
Melamine concentrations (mg kg^{-1}) in kitchen plastic ware samples obtained using the proposed HPLC–Fluo method and the HPLC–UV method.

Sample	Melamine concentration (mg kg^{-1})[a]	
	HPLC–FLUO	HPLC–UV
1	2.5 ± 0.58	2.7 ± 0.22
2	0.56 ± 0.07	< LOQ
3	0.26 ± 0.05	< LOQ
4	28 ± 0.38	29 ± 0.12
5	86 ± 4.1[b]	78 ± 2.2[b]
6	0.40 ± 0.03	< LOQ
7	0.90 ± 0.12	< LOQ
8	4.4 ± 0.13	4.2 ± 0.21
9	2.0 ± 0.33	< LOQ
10	23 ± 0.79	29 ± 0.18

[a] Mean ± SD (n=3).
[b] The sample was diluted (1:5) and reanalyzed.

were observed in these spectra demonstrating also the very good selectivity of the HPLC–Fluo method and its ability to identify the analyte of interest in the studied samples (Fig. 7).

Ten different samples of kitchen plastic ware were submitted to the extraction processes described above (Section 2.5), and analyzed simultaneously by HPLC–Fluo and HPLC–UV (Table 2). The extract of sample 5, which exceeded the linear range of both methods, was diluted (1:5) and reanalyzed. Samples 2, 3, 6, 7 and 9 showed no signals under HPLC–UV but were detected by HPLC–FLUO. The results obtained reaffirm the best sensitivity of the proposed method because most positive samples showed no signals under the HPLC–UV method. The concentrations that were larger than the LOQ of this method were in good agreement with those found by HPLC–Fluo.

4. Conclusions

The newfound natural luminescent properties of melamine in aqueous media without addition of any other reagent or derivatization were demonstrated in this study. These properties led to the development of new HPLC–Fluo method that was applied in the determination of melamine in samples of kitchen plastic ware made of melamine–formaldehyde resin.

The proposed HPLC–Fluo method is simple, precise and accurate. Analytical figures of merit were adequate for the determination of trace amounts of melamine with a low LOQ (0.027 μg mL^{-1} or 0.14 mg kg^{-1}) that allowed the detection of plastic ware contamination by melamine at trace level.

The method was successfully applied to the analysis of aqueous extracts of kitchen plastic ware made of melamine–formaldehyde resin. The concentrations found in these samples demonstrated that the migration of residual melamine monomer from these utensils might occur, leading to food contamination.

In practical terms, the results presented here are an alert to the society because they show that microwave heating of food and beverages in plastic ware made of melamine–formaldehyde resin, even under mild conditions, releases melamine. This practice would allow human exposure to this compound and therefore, would be potentially harmful to human health.

As far as we are aware, this is the first demonstration of the natural fluorescence of melamine and its analytical application.

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References

[1] U.S. International Trade Commission, Melamine Institutional Dinnerware from ChinaIndonesia, and Taiwan, Publication, Washington DC, 1996 (20436).
[2] M. Kohlmayr, G. Zuckerstätter, A. Kandelbauer, J. Appl. Polym. Sci. 124 (2012) 4416–4423.
[3] S. Jahromi, Macromol. Chem. Phys 200 (1999) 2230–2239.
[4] K.H. Lund, J.H. Petersen, Food Addit. Contam 23 (9) (2006) 948–955.
[5] H. Ishiwata, T. Inoue, A. Tanimura, Food Addit. Contam 3 (1) (1986) 63–69.
[6] E.L. Bradley, V. Boughtflower, T.L. Smith, D. Speck R, L. Castelo, Food Addit. Contam 22 (6) (2005) 597–606.
[7] E.L. Bradley, L. Castelo, J.S. Dia, I. Ebner, K. Ehlert, R. Helling, S. Koster, J. Vazamento, K. Pfaff, Food Addit. Contam. 27 (12) (2010) 1755–1764.
[8] Z. Chik, D.E. Mohamad Haron, E.D. Ahmad, H. Taha, P.M. Mustafa, Food Addit. Contam. 28 (7) (2011) 967–973.
[9] Federal Institute for Risk Assessment (BfR), 2011, ⟨http://www.bfr.bund.de/en/press_information/2011/11/cooking_spoons_and_crockery_made_of_melamine_resin_are_not_suited_for_microwaves_and_cooking-70465.html⟩, access date: July 2013.
[10] A.K. Hau, T.H. Kwan, P.K. Li, J. Am. Soc. Nephrol 20 (2009) 245–250.
[11] J.R. Ingelfinger, N. Engl. Med 359 (26) (2008) 2745–2748.
[12] WHO, Toxicological and Health Aspects of Melamine and Cyanuric Acid, Report of a WHO Expert Meeting In collaboration with FAO Supported by Health Canada, Geneva, 2009.
[13] J.L. Dorne, D.R. Doerge, M. Vandenbroeck, J. Fink-Gremmels, W. Mennes, H.K. Knutsen, F. Vernazza, L. Castle, L. Edler, D. Benford, Toxicol. Appl. Pharm 270 (3) (2013) 218–229.
[14] Y. Wu, Y. Zhang, Food Chem. Toxicol 56 (2013) 325–335.
[15] F. Sun, W. Ma, L. Xu, Y.Y. Zhu, L. Liu, C. Peng, L. Wang, K. Kuang, C. Xu, Trend. Anal. Chem 29 (11) (2010) 1239–1249.
[16] S.A. Tittlemier, Food Addit. Contam 27 (2) (2010) 129–145.
[17] T. Sugita, H. Ishiwata, K. Yoshihira, A. Maekawa, Bull. Environ. Contam. Toxicol. 44 (1990) 567–571.
[18] G. Venkatasami, J.R. Sowa , Anal. Chim. Acta 665 (2010) 227–230.
[19] Grace Davison Discovery Sciences, HPLC and UHPLC Methods for Melamine, ⟨http://www.discoverysciences.com/uploadedFiles/Vision_HT/M287_Melamine_AppNote_LR.pdf⟩, 2009, access date: january 2013.
[20] M.M. Deabes, R. El-Habib, J. Environ. Anal. Toxicol 2 (4) (2012) 2–4.
[21] T.T. Chang, Anal. Chem 66 (1994) 3267–3273.
[22] S. Turnipseed, C. Casey, C. Nochetto, D.N Heller, Determination of Melamine and Cyanuric Acid Residues in Infant FormulaFDA, LIB (2008) 24.
[23] M. Smoker, A.J. Krynitsky, Interim Method for Determination of Melamine and Cyanuric Acid Residues in Foods Using LC–MS/MSFDA, LIB (2008) .
[24] P. Lutter, M.C. Savoy-Perroud, E. Campos-Gimenez, L. Meyer, T. Goldmann, M.C. Bertholet, P. Mottier, A. Desmarchelier, F. Monard, C. Perrin, F. Robert, T. Delatour, Food Control 22 (2011) 903–913.
[25] Y.Y. Zhou, J. Yang, M. Liu, S.F. Wang, Q. Liu, J. Lumin. 130 (2010) 817–820.

[26] Z. Guo, P. Gai, T. Hao, S. Wang, D. Wei, N. Gan, Talanta 83 (2011) 1736–1741.
[27] L. Guo, J. Zhong, J. Wu, F.F. Fu, G. Chen, Y. Chen, X. Zheng, S. Lin, Analyst 136 (2011) 1659–1663.
[28] D. Xiang, G. Zeng, K. Zhai, L. Li, Z. He, Analyst 136 (2011) 2837–2844.
[29] A.M. Othman, J. Anal. Chem 67 (2012) 349–353.
[30] J. Rima, K. Assaker, F.E. Omar, C. Karim, Talanta 116 (2013) 277–282.
[31] S.G. Schulman, Fluorescence and Phosphorescence Spectroscopy: Physicochemical Principles and Practice, first ed., Pergamon Press, New York, 1977.
[32] National Institute of Metrology, Quality and Technology (INMETRO), Guidance on Validation of Analytical Methods. DOQ-CGCRE-008, Brazil, 2010.
[33] S.V.C. Souza, R.G. Junqueira, Anal. Chim. Acta 552 (2005) 25–35.