Talanta 82 (2010) 1543–1547

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Monitoring of melamine contamination in fat watery milk by the photoluminescence analysis

T. Serdiuk [a,*], V.A. Skryshevsky [a], M. Phaner-Goutorbe [b], E. Souteyrand [b]

[a] Institute of High Technology, Taras Shevchenko National University of Kyiv 64, Volodymyrska St., 01601 Kyiv, Ukraine
[b] Institut des Nanotechnologies de Lyon UMR 5270, Ecole Centrale de Lyon, 36 avenue Guy de Collongue 69134 Ecully, France

ARTICLE INFO

Article history:
Received 2 April 2010
Received in revised form 7 July 2010
Accepted 19 July 2010
Available online 27 July 2010

Keywords:
Melamine
Milk
Photoluminescence

ABSTRACT

A photoluminescence method to detect the toxic melamine contamination in fat watery milk has been proposed. Despite the quite different luminescence origins of milk and melamine patterns, their wide emission spectra under UV excitation are similar and in the range of 2.2–3.5 eV. The complex milk photoluminescence spectrum composed of riboflavin, furosine, lactulose, Vitamin E and tryptophan emitting species can be modified if milk pattern is undergone by acid treatment (for example, in vinegar). At the same time the melamine emission is not subjected to any modification in vinegar. It allows quantitatively discriminating the melamine contamination in milk in linear range, at least, 0.05–7 g/l from different photoluminescence spectra of milk (water) with and without melamine. Limit of melamine detection achieves 0.01 g/l.

1. Introduction

Melamine is an organic base and a trimer of cyanamide, with a 1,3,5-triazine skeleton and with the general formula of $C_3N_6H_6$ [1]. Melamine is a colorless crystal. Industry uses it to produce melamine resin, plastic and a very durable thermosetting plastic and melamine foam. Toxic melamine in milk and dairy products by certain unfair manufacturers has been added for imitation of high protein level in milk [1]. Ingestion of melamine may cause reproductive damage or bladder and kidney stones, which can lead to bladder cancer [2]. In Europe, the addition of melamine in food and animal feed is prohibited. The World Health Organization calls the toxic milk crisis which stemmed from China, one of the largest food safety events the agency has had to deal with in recent years. The European Commission therefore decided that food or feed containing milk products should be checked. The European Union set a standard for acceptable human consumption of melamine at 0.2 mg per kg of body mass. To minimize the risk of similar incidents in future, the European Commission and its members vigilantly monitor the presence of contaminants in food. The World Health Organization's food safety director estimated that the amount of melamine a person could stand per day without incurring a bigger health risk, the "tolerable daily intake" (TDI), was 0.2 mg per kg of body mass. So, it is very important to control the presence of melamine in dairy products. However, this task presents certain problems. Well-known food tests to determine the protein level are based on studying nitrogen level [1]. Since the molecule of melamine contains six atoms of nitrogen, melamine itself behaves like protein in these tests. That is why the melamine control test should be built upon another principle.

There are few analytical technical methods currently being used to detect the melamine at the laboratory conditions. The first one is a liquid chromatography method, described in [3]. A sensitive chemiluminescence method based on a luminol–myoglobin system is also proposed for the determination of melamine in dairy products [4]. High-throughput trace melamine analysis in complex mixtures is proposed in [5]. Ambient ionization using a low-temperature plasma probe combined with tandem mass spectrometry is used in this work. However, all these methods need costly laboratory equipments and cannot be applied for rapid-analysis in domestic conditions. So, there is an urgent need to create new effective analytical equipments, which are rapid, relatively low-cost and can be applied in fundamental research and in the factory as lab-on chip to monitor the falsifications with milk.

The alternative simple method for melamine detection based on photoluminescence technique is being discussed in this work. We used photoluminescence spectroscopy method since both milk [6–8] and melamine [9–14] display efficient emission in visible spectra region under UV excitation.

2. Materials and methods

Watery milk, milk powder and melamine powder were studied. Watery fat milk (3.2% of fat) without any thermal elaboration was

* Corresponding author.
E-mail addresses: ts@univ.kiev.ua, tanyshka1989@bigmir.net (T. Serdiuk).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.07.046

used in our work. Melamine probes (Russian GOST 7579-76) with 99.8% purity level were taken from routine delivery for chemical industry. We added melamine in watery milk sample (up to 10 g melamine on 1l of milk or distillate water). Since the melamine dissolves in water badly, to improve the dissolving of melamine we used the ultrasonic bath. Procedure for dissolving melamine was continued 10 min in bath with power of 50 W to fully dissolve melamine.

The samples in the form of solutions or powders are kept in special quartz cell. Photoluminescence spectra were studied at the excitation with a pulse of nitrogen laser ($\lambda = 337.1$ nm, $\tau = 8$ ns, 20 kW power in pulse) at room temperature. The light spot with diameter of 1 mm was focused on the sample surface. The sample emission was tended toward monochromator (MS2004, SOLAR T2) via optical fibre and registered by photomultiplier (HAMAMATSU C6270) and analog-to-digital board (up to 1 GHz sample rate), then the signal was processed by PC. Research facility used for photoluminescence analysis is similar to the one described in [15].

3. Results and discussion

The solid powder of melamine is characterized by complex strong blue-green photoluminescence at room temperature in 2.2–3.5 eV spectral regions (Fig. 1(a)). Its form is closely similar to the previously reported one [6–8]. It allows us to conclude that despite the absence of usage of the chemically pure melamine substance, the observed photoluminescence spectrum is conditioned, namely, by pure melamine. The emission at 3.29 eV comes from triazine moieties in the melamine molecule, an emission peak at 3.09 eV is considered to be derived from the electronic ground state associated with the form in the triazine polymer. This indicates that a small amount of the triazine polymer existed in the raw melamine powder. The 2.79 eV peak corresponds to the presence of transforming melamine into monoclinic crystal consisting triazine polymers with sp^2 C=N bonding [6]. Then photoluminescence spectrum of melamine can be fitted as a sum of 3 Gauss components that have different features. Peak maximum, intensity, and full width in half maximum (FWHM) for each component, accordingly, were found by Curve Fitting Toolbox of MatLab in own confidence interval. Average coefficients of mathematical model that correspond to energy, FWHM and intensity of all components are shown in Table 1.

Complex strong green-blue photoluminescence is a representative of the watery milk at room temperature. Milk contains different biological and chemical components, which have different chemical structures and emission properties. There are tryptophan (violet region), lactulose, furosine, Vitamin A (blue photoluminescence), riboflavin (green spectrum region) and other species. As a result, the obtained spectrum of watery milk can be characterized at least by a few Gauss components. Mathematical Packet also was used to find the maximum peak, the intensity of maximum, and the FWHM of it (Table 2). At least, the model containing 3 Gauss components fits well to the obtained experimental results, and this model corresponds to the nature of milk photoluminescence. So, the first peak of 3.21 eV energy corresponds to proteins in milk. The most important of them is tryptophan that has sample emission spectrum [9–11]. The second peak with energy of 3.05 eV correspond to photoluminescence of lactulose, furosine and Vitamin A. Each of the spectra superimposed was presented as one peak. The third maximum location at energy of 2.64 eV satisfies riboflavin

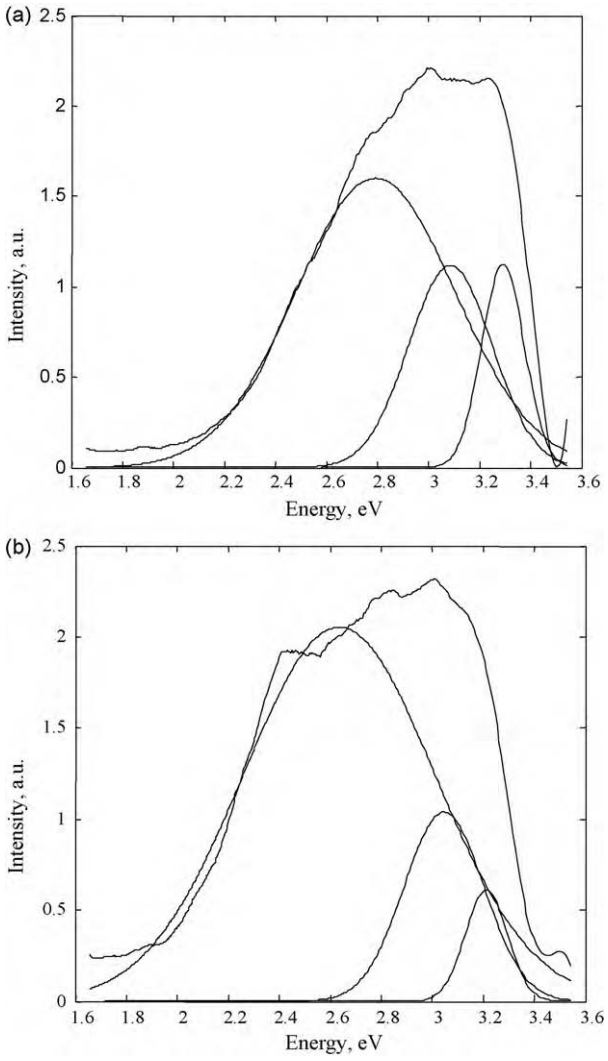

Fig. 1. Photoluminescence spectra of melamine (a) and watery milk (b) with the Gauss components.

Table 1
Description of spectrum components for melamine powder.

No. of max	Peak position (eV)	Intensity (a.u.)	FWHM (eV)	Relative efficiency (a.u.)	Description	Ref.
1	2.79	1.60	0.88	1.41	Triazine polymers with sp^2 C=N bonding	[6]
2	3.09	1.05	0.46	0.48	Triazine polymer	[6–8]
3	3.29	1.12	0.24	0.27	Triazine moieties	[6–8]

Table 2
Description of spectrum components for watery milk.

No. of max	Peak position (eV)	Intensity (a.u.)	FWHM (eV)	Relative efficiency (a.u.)	Description	Ref.
1	2.64	2.05	1.06	2.17	Riboflavin	[12–14]
2	3.05	1.04	0.44	0.46	Furosine, lactulose, Vitamin A	[12–14]
3	3.21	0.61	0.24	0.15	Tryptophan	[9–11]

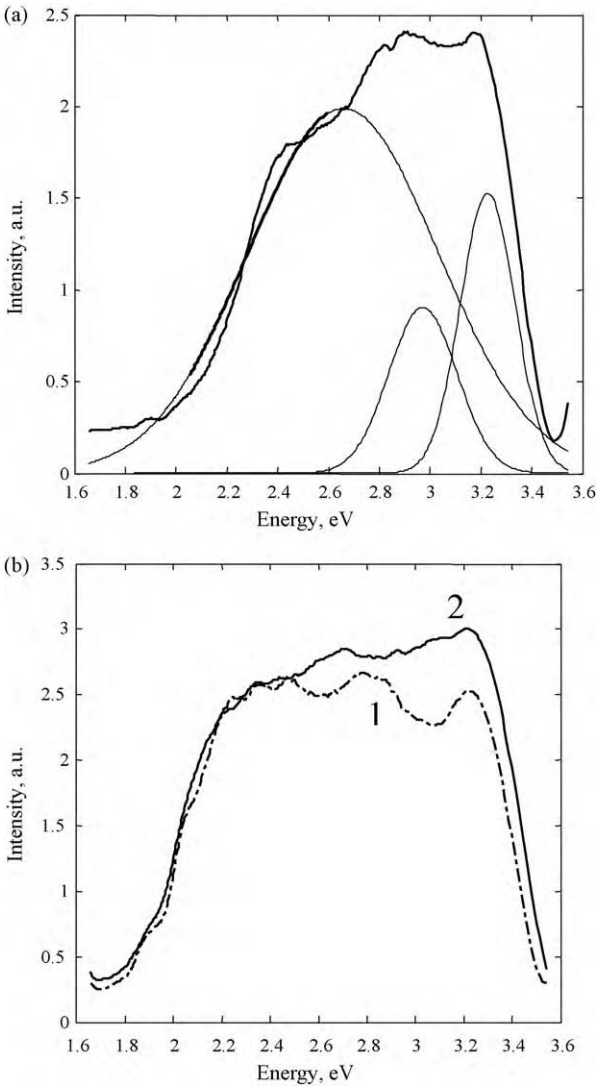

Fig. 2. Photoluminescence spectrum of: (a) sample containing watery milk and melamine (10 g/l) with the Gauss components, (b) milk powder (1) and mixture of milk and melamine powders in 1:1 proportion (2).

[12–14] (Fig. 1(b)). The photoluminescence of watery milk sample containing melamine is shown in Fig. 2(a). So, obtained spectrum of substance was also analyzed by Mathematical Packet. It again can be fitted by 3 Gauss components. Maximum, FWHM and intensity of peaks are shown in Table 3.

Photoluminescence spectra of melamine and milk are wide and have similar intensity in general, and they have a little different distribution between separate components. For both substances, short wavelength peak is, at least, 3–5 times more intensive than long wavelength. The preparation of milk and melamine mixture (in saturated melamine solubility) changes drastically neither the intensity of distribution between short/long wavelength peaks nor their energy position. It is not surprising, because there are very close luminescence parameters for both matters (Fig. 1(a) and (b)). The same result was obtained at the photoluminescence measurements of powder milk with and without melamine: the powder milk and melamine mixture display close photoluminescence properties (Fig. 2(b)).

Table 3
Description of spectrum components for watery milk with melamine (10 g/l).

No. of max	Peak position (eV)	Intensity (a.u.)	FWHM (eV)	Relative efficiency (a.u.)
1	2.66	1.99	1.06	2.11
2	2.98	0.90	0.38	0.34
3	3.23	1.53	0.30	0.46

As we can see, the spectra of milk and milk with melamine are spectrally wide and similar in general, that is why we should look for a way to improve the spectral contrast, for instance via difference in photoluminescence yield of these matters. Our approach is based on the application of supplementary treatment of milk–melamine solution which results in the selective emission quenching of one or a few emitting species. For example, for the melamine identification in milk, a precipitation of proteins followed up by filtration of non-homogeneous substance was used. The precipitation of protein in milk by vinegar was used in this work to detect melamine in 350–400 nm wavelength regions, where melamine and protein of milk [6–8] have had approximate maximum [12–14]. The vinegar was added to milk and milk with melamine. The vinegar was chosen for the precipitation of proteins as a very simple substance that is safe for simple, but not laboratory, usage. Influence of vinegar presence on melamine emission is faint. After the addition of vinegar, the milk is soured and divided into two fractions. If the proteins are precipitated and much part of organic substances remains in curdle mass, the melamine stays in colorless serum. Obtained solutions were filtered through filtered paper and only serum was used to detect melamine.

Photoluminescence spectrum of milk sample with vinegar after filtration is shown in Fig. 3(a). Photoluminescence of milk with vinegar is less intensive due to quenching, especially for 3.21 and 2.64 eV peaks associated, first of all, with milk proteins. It allows us to reduce the milk contribution to the general emission spectra of studying patterns with melamine. Dependence of melamine contained in general photoluminescence spectrum is also shown in Fig. 3(a). Proportions between milk and vinegar were the same in all samples (20 ml milk and 20 ml of vinegar were taken). The well-detected melamine concentration lies in the range from $C = 10$ to 0.05 g/l at the luminescence yield measurement accuracy of over 5% (Fig. 3(b), curve E_1). In all spectral regions of melamine emission the concentration dependence has the same behavior – the emission yield increases linearly from 0.05 to 7 g/l concentration with the following small saturation at the bigger melamine concentration (Fig. 3(b)). At that time, the limit of detection (LOD) can achieve 0.01 g/l. The sensitivity ($S = (\Delta I/I)/C$) of the melamine detection is 0.9, 0.4 and 0.2 1/(g/l) for peak with energies of 3.29, 3.09 and 2.79 eV, respectively.

The maximal sensitivity is achieved at the analysis of melamine emission component with energy 3.29 eV that corresponded to amino-groups, since in control pure milk sample after protein precipitation the component with the same energy disappeared. In samples that contained milk and melamine, by the increase of melamine concentration the photoluminescence intensity with energy near 3.29 eV also is increased up to the value that corresponds to pure melamine sample. It means that using proposed method of photoluminescence intensity analysis in this spectral region, we can detect melamine in.

Some other specific amino-group if any is in milk (besides protein that precipitated in acid) also can give some emission peaks in this spectral region. Besides, the presents of not native to milk substance contained Nitrogen also can give illusion of "high proteins level" in dairy products. High selectivity possibility of proposed melamine detection method is ensured using complex analysis of photoluminescence spectrum due to analysis of all emission components with energy values 3.29, 3.09 and 2.79 eV. In this case, we can be sure that amino-groups of falsification substance really corresponded to melamine, since photoluminescence of the three components gives specific form of melamine spectrum.

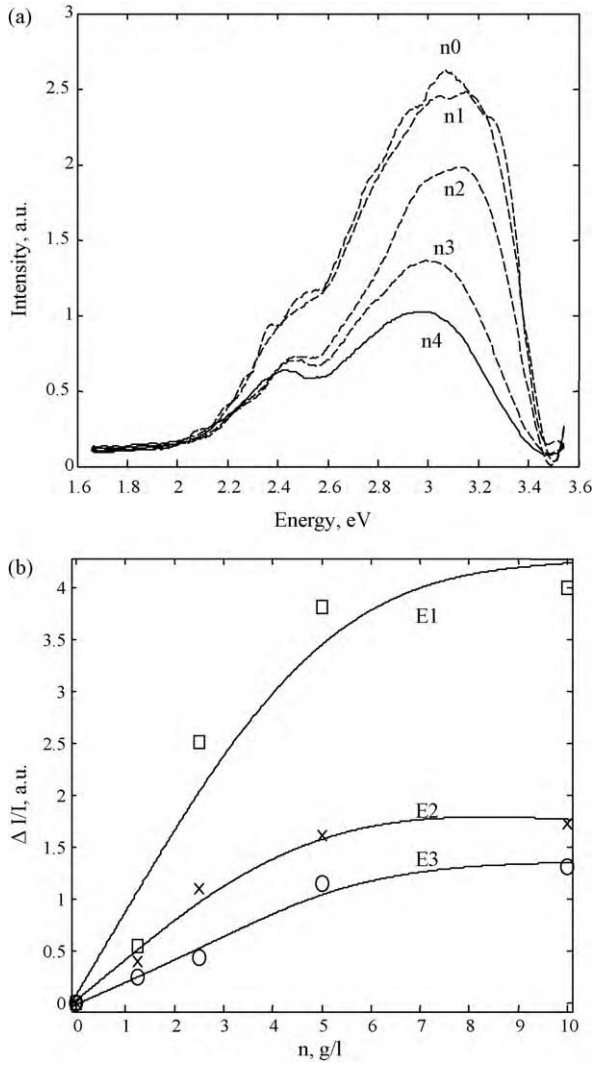

Fig. 3. (a) Photoluminescence spectrum of filtered mixture containing milk, vinegar and melamine with different levels of the melamine concentration: $n_0 = 10$ g/l, $n_1 = 5$ g/l, $n_2 = 2.5$ g/l, $n_3 = 1.25$ g/l, and filtered mixture contained milk and vinegar only ($n_4 = 0$ g/l), (b) relative photoluminescence intensity of mixture containing milk, vinegar and melamine versus concentration levels of melamine for energies: $E_1 = 3.29$ eV, $E_2 = 3.09$ eV, $E_3 = 2.79$ eV.

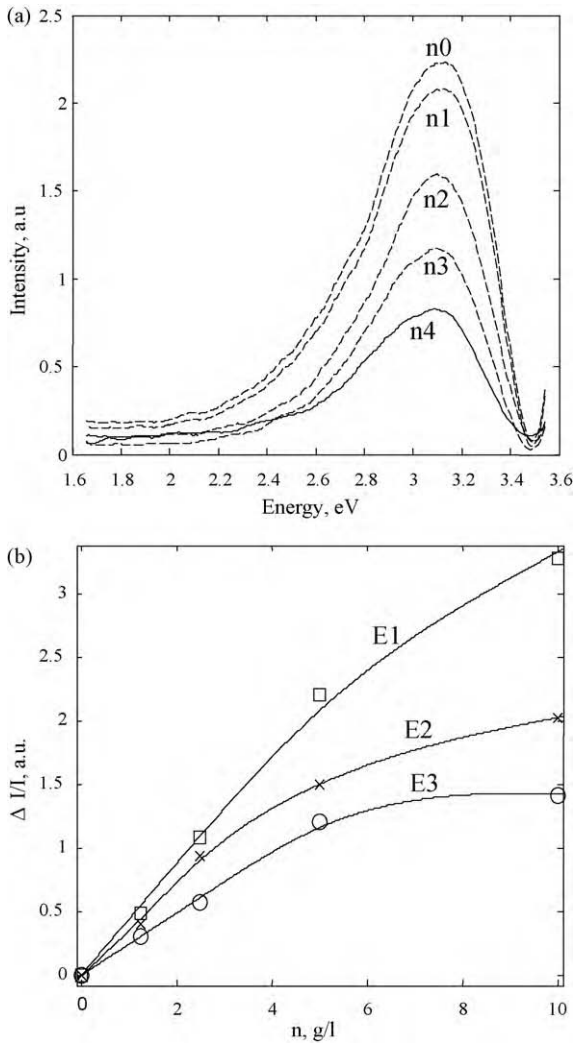

Fig. 4. (a) Photoluminescence spectrum of water containing different levels of the melamine concentration: $n_0 = 10$ g/l, $n_1 = 5$ g/l, $n_2 = 2.5$ g/l, $n_3 = 1.25$ g/l, and without melamine ($n_4 = 0$ g/l), (b) relative photoluminescence intensity versus concentration levels of melamine for energies: $E_1 = 3.29$ eV, $E_2 = 3.09$ eV, $E_3 = 2.79$ eV.

High reproducibility of proposed method is ensured by weak impact of milk sorts on the obtained dependences. Proposed method has several advantages compared with existing method. First of all the melamine determination procedure is simple. The method does not require any expensive specific reagents (only vinegar), and has high recoveries and can be used reused, such analysis is really very rapid (few minutes on one procedure). This relative method is low-cost compared with other by few orders. All existing methods have a narrow linear range and cannot determinate high melamine concentration that was found in dairy products in "Melamine China scandal" (to 3 g/l). Linear range of the photoluminescence method is from 0.05 to 7 g/l. Proposed method based on photoluminescence analysis allows to determine melamine concentration close to the boundary safe concentration for human. LOD of proposed method is 0.01 g/l. LOD of liquid chromatography method is 0.1 mg/l but linear range is only from 0.2 to 5 mg/l [3]. LOD of the luminol–myoglobin method is 3 pg/ml, but linear range is only from 10 pg/ml to 5 ng/ml [4] that is not optimal to real demands. High-throughput trace melamine analysis also has very good LOD mean of 100 pg with linear range of 10–2500 ng/l [5]. Despite that these methods give smaller LOD, such small LOD meaning is not needed for practice (according to safe melamine concentration level for human).

However, to comply with the request of World Health Organization, it is desirable to lower the detecting threshold of photoluminescence methods, at least, on few times. It can be achieved by either the improvement of the photoluminescence measurement precision (it means the luminescence yield measurement accuracy should be over 0.5% due to linear behavior of the concentration dependence) or the reduction of background emission. It is obvious that under UV illumination the water–milk solution generates the background emission. Even after suppressing the milk emission till they vanish, there is still the emission of water due to the presence of some species [16]. Appearance of maximum intensity near 425 nm is relevant to the trace amount of impurities adsorbed at the bubble/water interface. The presence of gas nanobubbles (emission around 300 nm) in non-outgased water samples was identified using dynamic light scattering. Fig. 4(a) shows the emissions of water containing melamine at the same concentrations as in watery milk that discussed before (Fig. 3(a)). As can be seen, the background emission of water is sufficient that results in the same sensing in melamine detection as in milk treated by vinegar (Fig. 4(b)). Appli-

cation of non-luminescent deionized water [16] should reduce the detecting threshold level of melamine in water, however, likely, the deionized water is not used in real conditions of counterfeit production with melamine additives instead of natural milk.

The method based on the analysis of photoluminescence spectra is considered to detect the unexpected melamine contamination in fat watery milk. Since the quantum yield and photoluminescence spectra for milk and melamine at the equal concentration are the same we propose an approach based on the suppression of milk emission. To improve the spectral contrast we consider supplementary treatment by vinegar, since the treatment of milk/melamine solution by vinegar quenches the milk emission and does not impact on melamine one. It has given us good results in samples of milk with and without melamine. Vinegar used for the precipitation of protein made this analyze safe and simple. The usage of proposed method give base for creation a simple sensor with photoluminescence transduser for industry or even for home in order to detect such dangerous food falsification as the addition of melamine in milk in the nearest future.

References

[1] Report of WHO Expert meeting in collaboration with FAO supported by Health Canada, Toxicological and Health Aspects of Melamine and Cyanuric Acid, Geneva, Switzerland, 2008.
[2] A.K. Hau, T.H. Kwan, P.K. Li, J. Am. Soc. Nephrol. 20 (2) (2009) 245–250.
[3] US Food and Drug Administration, LIB 24 (4421) (2008).
[4] Z. Wang, D. Chen, X. Gao, Z. Song, J. Agric. Food Chem. 57 (9) (2009) 3464–3469.
[5] G. Huang, Z. Ouyang, R. Graham Cooks, Chem. Commun. 10 (2009) 556–558.
[6] D.L. Yu, J.L. He, Z.Y. Liu, B. Xu, D.C. Li, Y.J. Tian, J. Mater. Sci. 43 (2) (2008) 689–695.
[7] M. Hasegava, A. Ishii, K. Habu, H. Ichikawa, K. Maeda, S. Kishi, Y. Shigesato, Sci. Technol. Adv. Mater. 7 (2006) 72–76.
[8] A. Saha, B. Roy, A. Garai, A.K. Nandi, Langmuir 25 (5) (2009) 8457–8461.
[9] J.T. Vivian, P.R. Callis, Biophys. J. 80 (2001) 2093–2109.
[10] G.D. Fasman, Handbook of Biochemistry and Molecular Biology, Proteins I, 3rd ed., CRC Press, 1976, pp. 183–203.
[11] D.M. Corgliano, H. Hofstetter, O. Hofstetter, BMG Labtech. 162 (2007) 1–6.
[12] S. Herbert, A. Riaublanc, B. Bouchet, D.J. Gallant, E. Dufour, J. Dairy Sci. 82 (10) (1999) 2056–2062.
[13] R.R. Gangidi, L.E. Metzger, J. Dairy Sci. 89 (11) (2006) 4105–4113.
[14] A. Kulmyrzaev, E. Dufour, Lait 82 (2002) 725–735.
[15] A. Benilov, I. Gavrilchenko, I. Benilova, V. Skryshevsky, M. Cabrera, Sens. Actuators A 137 (2007) 345–349.
[16] P. Vallee, J. Lafait, P. Mentre, M.-O. Monod, Y. Thomas, Langmuir 21 (6) (2005) 2293–2299.