Visual-test and Sorption-spectrophotometric Determination of Melamine

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Melamine may have toxic effects on humans and animals. It is well known that melamine accumulates in the body and causes reproductive damages, forms bladder or kidney stones, which can lead to bladder cancer. Trace amounts of melamine at ppm levels may occur in certain food commodities due to its migration from melamine-containing disposable tableware plastics. It was intentionally adulterated to milk products to show a false increase in protein concentration. Considering these facts there is a need for establishing sensitive and reliable methods of melamine determination. As uncostly, rapid and selective melamine detection methods are highly required, the hyphenated sorption-spectrophotometric and visual test methods seem to be perspective candidates. In the present work the optimal conditions of sorption concentration of melamine from aqueous solutions onto the silica gel surface were studied. The calibration graph for the sorption-spectrophotometric method is linear in 0.02 – 9.8 $\mu mol \cdot L^{-1}$ mg L^{-1} melamine concentration range. Proposed method allows naked-eye monitoring of biological samples.

Introduction

Melamine (1,3,5-triazine-2,4,6- triamine, MEL) is mainly used to produce melamine-formaldehyde resins for manufacturing of kitchenware in particular. Unfortunately, MEL migrated from tableware plastics into beverage under acidic conditions [1]. MEL was reported illicitly added to a number of different types of animal and human-food sources to increase the apparent protein content [2]. When MEL exceeds the EU safety limit (0.5 ppm in [3]), it may trigger renal injury [4], lead to reproductive damage, bladder cancer, or death [5-8]. Therefore, considering the melamine contamination in food and beverages, there is a need for establishing sensitive and reliable methods that are capable of screening samples and confirming the presence and quantities of melamine.

Usually MEL in feed and food [2] are analyzed by liquid chromatography [9 -11], liquid and gas chromatography/mass spectrometry [12, 13]. These methods are sensitive (quantification limit 0.01 – 0.05 $mg \cdot kg^{-1}$) but require the use of expensive equipment, highly trained personnel and carrying out long-term sample preparation. Cheaper and more express methods [2], such as capillary zone

electrophoresis, voltammetry, ionometry, chemiluminescence, etc., are required specific equipment and they are inconvenient for using in most conventional laboratories.

Rapid screening methods [14] like enzyme-linked immunosorbent assay (ELISA) are faster and less expensive than instrumental methods, but for their lack of selectivity the positive samples from these screening must be confirmed by selective methods. The aggregation of citrate-stabilized gold nanoparticles (AuNP) in the presence of MEL causes the dramatic colour changes and could be visually detected [15]. However, specificity of the method is clear.

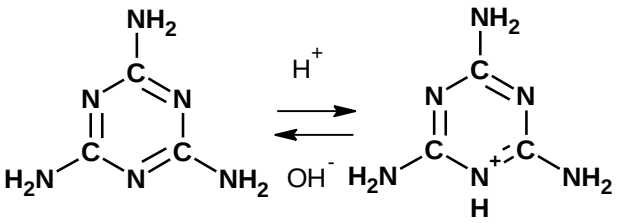
Because of the common availability of the instrumentation, the simplicity of procedures, precision and accuracy of the technique, spectroscopy is a convenient and widely used method for quantitative analysis. In recent years a few of papers about optical methods of determining MEL have been reported. A spectrophotometric method based on Mannich reaction between MEL and a mixture of formaldehyde and uranine [16], fluorescence methods using cucurbit[7]uril(CB)sensor [17] and $[Ru(CO)_2(L)]$ (where L – anion of tetradentate Schiff base) [18] were developed. However, complicated pre-concentration procedure limits practical applications of these methods. The method without long-term pre-separation step was developed in [19]. It based on rank annihilation factor analysis of pH-gradual change-UV spectral data (pH-spectra).

The other method to improve selectivity and sensitivity of spectrophotometry is its combination with pre-concentration step [20]. Therefore, the hyphenated sorption-spectrophotometric (SSPh) and visual test (VT) methods seem to be perspective candidates as uncostly, rapid and selective melamine detection methods. In the present work the optimal conditions of sorption concentration of melamine on the silica gel surface from aqueous solutions were optimized using spectrophotometry and naked-eye monitoring.

Results and discussion

Mineral sorbents, silica particularly, offer some distinct advantages over the organic polymer supports: short equilibration time and excellent swelling resistance in different solvents. In the present work mesoporous silica SG-60 (SG) was selected as a sorbent because of its high sorption ability to adjust pore sizes and ability to easily regeneration.

The adsorption of MEL onto the silica gel surface from aqueous solutions in the pH range 1.0 -7.0 was studied. It was found that ≥ 85% of MEL is adsorbed in the pH range 2.5 - 5.0. Under such conditions melamine forms conjugated acid $HMEL^+$ (**Figure 1**) as a weak base with pK_a 5.05 [21].



MEL HMEL$^+$

Figure 1. Molecular structure of melamine and its conjugated acide.

The further experiments were conducted at the pH 3.5±0.1, when MEL recovery exceeds 90%. The kinetic experiments showed that the equilibrium of the adsorption of MEL onto the SG surface was reached in 15 min.

The conditions for the determination of MEL were optimized. For this purpose the adsorption of melamine as a function of solution volume/mass of sorbent was studied. The maximum distribution coefficient (D) was found to be $3.8 \cdot 10^3$ ml·g^{-1} at V=100 ml and m=0.05 g.

The absobance spectra of SG treated with the solution of MEL at different concentrations are presented in **Figure 2**. The maximum difference in the spectra of SG (**Figure 2**, curve 1) and SG treated with the solution of MEL (**Figure 2**, curve 2-4) is observed at λ=280 nm and caused by light absobance of MEL grafted on the surface. It is shifted bathochromically by 10 nm compared with one in solution [19]. This fact may be the result of polarizing effect of the silica surface.

It was shown that the intensity of signal at 280 nm increased in proportion to the melamine concentration in solution. The equation of the calibration graph was

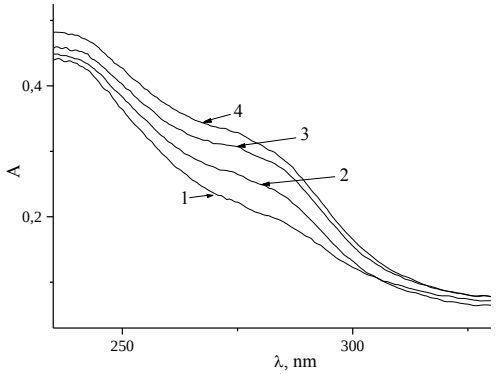


Figure 2. Absorbance spectra of SG (1) and SG with MEL adsorbed (2-4). pH 3.5, V = 10 ml, m = 0.05 g, C_{MEL} (μmol·L^{-1}): 0.2 (2), 0.5 (3), 1.0 (4).

$A_{280}=(0.004\pm0.002)+(0.24\pm0.02)\cdot C_{MEL}(\mu mol \cdot L^{-1})$ r = 0,990, the linear range was 0.02 – 9.8 μmol·L^{-1}. The detection limit (DL, blank + 3σ, where σ is the standard deviation of blank estimation) was 0,05 μmol·L^{-1}, two times better than one for the best analog [19].

As the vast majority of objects that require the melamine screening and quantification (biological fluids, food, drinks, plastic, soil, etc.) contain a number of organic compounds that absorb electromagnetic radiation in the UV region and can be adsorbed on the surface of silica, selectivity of MEL detection will be poor. Earlier [20] to avoid the interference of foreign substances the sorbent was treated with especial organic reagent, which selectively reacts with the immobilized analyte to form a colored compound. As melamine adsorbed on the silica gel surface in HMEL$^+$ form, anionic dyes seem to be perspective candidates. The properties of a number of chromophoric reagents have been tested. The best results were obtained when 1% solution of methyl orange (sodium4-[(4-dimethylamino)

phenyldiazenyl] benzenesulfonate, MO) was used. The results obtained are shown in **Figure 3.**

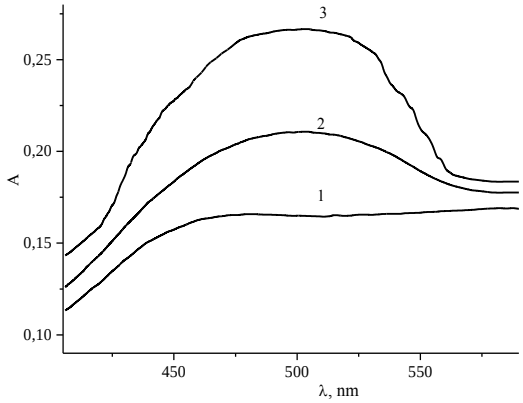


Figure 3. Absorbance spectra of SG (1) and SG with MEL adsorbed (2, 3) after treatment with MO. pH 3.5, m = 0.05 g, C_{MO} = 1%, C_{MEL} (μmol·L^{-1}): 0 (1), 0.5 (2), 1.3 (3).

It was shown that intensity of light absorption at 500 nm increased when melamine concentration grew. The colour scale for visual test determination in the range of 0.05 – 1.2 μmol·L^{-1} was developed. The scale was prepared as follows: 10 mL solutions of melamine containing 0; 0.05; 0.1; 0.2; 0.5 and 1.2 μmol·L^{-1} at the pH 3.5±0.1 were stirring with 0.05 g SG for 15 min. The sorbents were separated and dried. Then, the SG surface with adsorbed MEL was treated with 1.0 mL of 1% MO solution at the pH 3.5. The residue of reagent was washed out with nitric acid solution at the pH 3.5 and sorbent was dried. The colour of sorbent turned from pink to red while the concentration of MEL is increasing. Conditions for MEL VT-detection were optimized. For this purpose the MEL detection was studied in the mixture imitating the average composition of foodstuffs [22] as function of mass of sample and content of analyte. It was found that all components of mixture do not interfere with MEL under VT-detection, the DL was 0.007 mg·kg^{-1} (for 0.1 kg of sample mass and 0.055 μmol·L^{-1} MEL concentration). The comparison of developed and known VT-methods for the determination of MEL is represented in the **Table 1**.

Table 1.

Comparative description of visual test methods

Method	DL of MEL, mg·kg^{-1}	Reference
ELISA	0.009	[14]
AuNP	0.04	[15]
proposed	0.007	

ELISA - enzyme-linked immunosorbent assay

AuNP – visual test based on aggregation of citrate-stabilized gold nanoparticles

The data indicate that the test proposed is more sensitive.

Conclusions

In the present work the optimal conditions of adsorption pre-concentration of melamine from aqueous solutions onto the silica gel surface were optimized. The methods of sorption-spectrophotometric quantification and VT screening of melamine were developed. The sensitivity of the method proposed is better than those ones for known analogues and it lets to detect melamine in biological objects on tolerable daily intake level (0.2 mg per kg of body mass [2]).

Experimental part

All chemicals used in the experiments were of analytical grade. All solutions were prepared using distilled water. Melamine was purchased from Ukrorgsintez, Ukraine, SG-60 (surface area 480 $m^2 \cdot g^{-1}$, pore diameter 6.0 nm, particle size 60 μm) – from Merck, Germany.

The stock solutions of melamine (1.0 $mmol \cdot L^{-1}$) was prepared according to the conventional method and storage at 4^0C. Standard working solutions were prepared by appropriate dilution of the stock solution.

The absorbance spectra were recorded using UV/Vis spectrophotometer "Specord M-40" (Carl Zeiss Jena, Germany). A potentiometer model EV-74 with glass electrode (Gomel, Belarus) was used for pH measurements.

The batch technique for studying of melamine adsorption onto the SG surface was used. For this purpose weighted amount (0.0500 – 0.5000 g) of SG was stirred with aliquot (10.0 – 200.0 mL) of melamine solution (concentration $1.0 \cdot 10^{-3}$ – $1.0 \cdot 10^{-8}$ $mol \cdot L^{-1}$) at the pH 0.5 – 10.0 for 1 – 40 min. The residue of melamine in solution after sorption was controlled spectrophotometrically by own light absorbance of molecular form of melamine (**Figure 2**) at λ = 203 nm. Thus, before measuring all the pH value of solutions were adjusted to 3.0±0.1 units. The adsorption value (a, $\mu mol \cdot g^{-1}$) and distribution coefficient of MEL (D, $L \cdot g^{-1}$) were calculated according [20].

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