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ORIGINAL ARTICLE

Melamine migration measurement through spectrophotometry device and the effect of time and tableware type on it

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ABSTRACT

Melamine is an organic-based chemical material widely used in the production of tableware. Given the adverse effects of melamine on human health, melamine tableware can be a source for its introduction into the human body. The aim of this study was to use a simple method for monitoring the rate of melamine migration from the tableware to food and the effect of time and tableware on this migration. To measure the migration, spectrophotometry was used. The limit of detection (LOD) of the method was 0.2 (µg/ml), which is functional for measuring the rate of migration. The investigation of sample migration of melamine tableware revealed that migration has occurred across all samples. The rate of migration in all samples was less than the standard level of the European Union (30 µg/ml). Statistical analysis indicated that time is an important factor in melamine migration, which significantly increased ($p<0.05$) in 93% of cases with lengthening the contact time from 30 minutes to 90 minutes. The type of tableware (new or old) and production conditions (standard or non-standard) were found to significantly affect ($p<0.001$) the rate of migration. Statistical analysis of the results suggested that old tableware increased melamine migration in 41% of cases ($p<0.05$). Non-standard tableware significantly ($p<0.001$) increased the rate of migration and thus the effect of non-standard production on melamine tableware was more significant than the age of the tableware.

KEY WORDS: melamine; migration; spectrophotometry; tableware type

Introduction

Melamine is an organic-based chemical with 67% of its mass being composed of nitrogen. This material has a strong framework of 1,3,5 triazine connected to six hydrogen atoms, which can be replaced with other groups, and produces various compounds of melamine. One of these derivations are melamine thermoset resins widely used in plastic industries, chemical fertilizers, floor and wall coverings, textiles, adhesives, dyeing, pharmaceutical products, and food-related tableware (JR, 2008; Rima, 2013). The use of melamine and its material is very common in producing plastic tableware due to its good heat resistance, high durability, cheap cost, and coming in various and attractive types. Melamine tableware has turned to one of the most important and common ways of human contact with melamine (Richardson, 2004; Bradley, 2010).

Longtime contact with melamine through digestive, respiratory or dermal absorption can threaten human health causing kidney failure and probably cancer (Lynch, 2015). In low concentrations, this substance causes acute oral toxicity and in high concentrations it results in kidney diseases and even death, especially in newborns and children. The entry of melamine into the body by different food may lead to the formation of a complex with cyanuric acid, which is in the form of an insoluble crystal for urine pH and leads to kidney stones, urinary tract tissue pathologies, and ultimately urinary retention due to blockage of the urinary tract (Chansuvarn, 2013).

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There is no particular law for using melamine in non-plastic substances in Europe. Nevertheless, since melamine is one of the raw materials for producing plastic products, used for serving food, a special limit of migration has been considered as a standard. Specific Migration Limit (SML) for melamine has been determined as 30 µg/ml by plastic unit in the European Union. The value of SML is derived from daily tolerable amount for humans per kilogram of body weight (Commission, 2009; Lynch, 2015). Since digestive contact is an important way of human contact with melamine and melamine is used in the synthesis of food-related utensils, there is some concern about melamine migration from tableware to food, where the availability of this tableware for children is of particular concern (Chien, 2011). The primary migration of melamine from melamine tableware to food may be due to remaining monomers. However, secondary reasons might be due to the breakdown and decomposition of polymers. Notably, the major concern is related to this second type migration (Hsu, 2010).

Given the extensive use of melamine in food tableware for maintaining, transporting, and serving food materials, melamine migration to food has changed into a topic of discussion as an important problem in the health and safety of food materials. The necessity of measuring and monitoring the magnitude of melamine migration from tableware to food is felt in order to control this hazard in food materials. Spectrophotometric method, which is a simple, cheap, and available method for most laboratories, was used in this study for measuring the rate of melamine migration. To this end, the method was first validated. Then, the rate of migration was investigated along with the effect of time and type of tableware on this migration in terms of newness or oldness as well as the conditions of tableware production in terms of being standard and non-standard.

Materials and methods

To determine the rate of melamine migration from melamine tableware in this study, three standard brands were selected. They are prepared based on standard number 612 of Iranian national standards organization with the strongest sales in the market and as a result with the greatest consumption. A sample of melamine dish with no label and sign of production site was selected as the non-standard sample, while a sample of melamine tableware, which had been used for two years, was selected as the old sample for investigating the effect of oldness on melamine migration.

As food stimulants, distilled water and acid acetic 3% were selected to get into contact with melamine tableware. Simulants were contacted to melamine tableware at 90 °C for 30 and 90 minutes to determine the effect of time on melamine migration. The rates of melamine migration from melamine tableware to simulants were measured by spectrophotometry.

Migration measurement by spectrophotometry method

The method was based on the complexation of melamine with a mixture of formaldehyde and chemicals, including a ketone group. This complex was the result of Mannich reaction. Uranin was used as a ketone compound; By UV/VIS (200–400 nm) spectrophotometry device the quantitative value of melamine was measured (Rima, 2009 ; Rima, 2013).

Mannich reaction consists of amino alkylation of an acidic proton located next to a carbonyl functional group by formaldehyde and a primary or secondary amine or ammonia. The final product is a β-amino-carbonyl compound (Figure 1).

Materials and device

Melamine (Sigma, Germany, Lot number: 1422105v), uranin (C20H10Na2O5) purity 99.0% (Sigma,Germany), formaldehyde (Merck,Germany), deionized distilled water, acetic acid 3% (Merck, Germany), spectrophotometer UV/VIS (Perkinelmer, America), scale with microgram detection limit (Sartorius,Germany), ultrasonic device (Elma, Germany), sampler (Eppendorf, Germany).

Preparation of the solutions and drawing the calibration curve

Melamine stock solution with a concentration of 6.3 µg/ml was prepared along with one solution of uranin with a concentration of 6.3 µg/ml. This was followed by preparing pure formaldehyde, which was another component of the intended complex.

To draw the calibration curve, 10 solutions were mixed with different volumes of stock melamine (0.05–2 ml) with 0.5 ml of uranin solution as ketone and 1 ml of pure formaldehyde. Next, different volumes of deionized distilled water were added to each of the solutions to reach a final volume of 5 ml (Table 1). In the final solution,

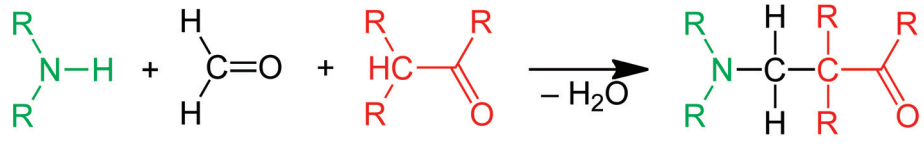

Figure 1. Mannich Reaction

Table 1. Concentration of different sections of melamine-uranin-formaldehyde complex for plotting the calibration curve.

Volume of Water (ml)	Volume of formaldehyde (ml)	Volume of Uranin [6.3 µg/mL] (ml)	Melamine Con (µg/ml)	Volume of melamine [6.3 µg/mL] (ml)
3.45	1	0.5	0.063	0.05
3.3	1	0.5	2.52	0.2
3.25	1	0.5	3.15	0.25
3	1	0.5	0.630	0.5
2.75	1	0.5	0.945	0.75
2.5	1	0.5	1.260	1
2.25	1	0.5	1.575	1.25
2	1	0.5	1.890	1.5
1.75	1	0.5	2.205	1.75
1.5	1	0.5	2.520	2

the concentration of uranin and formaldehyde was fixed, while the concentration of melamine varied between 0. 63 and 2.52 µg/ml. Then the solution was shaken and absorption of the solutions was measured by the device and the calibration curve was drawn. Three replications were considered for each concentration.

Validating the spectrophotometry method

To validate this method, the protocol proposed in the international conference of coordinated instructions was used for validating analytical methods including LOQ, LOD, linearity, and accuracy (Rima, 2009).

To obtain the value of LOD and LOQ, the value of signal was calculated by noise. To check the linearity, the relationship between the concentration and peak area was investigated. Ten different concentrations of melamine standard were prepared and read by the device and the correlation coefficient was determined.

To check the accuracy, iteration in one day (three iterations) and iteration in three consecutive days were used. At first volumes of 0.5, 1 and 2 ml of melamine standard solution with a concentration of 6.3 µg/ml were selected (the concentrations of .63, 1.26 and 2.52 µg/ml) and transferred to three test tubes. Then, 1 ml of formaldehyde and 0.5 ml of uranin were transferred to each of the test tubes and different volumes of deionized distilled water were added to each solution to reach the ultimate volume of 5 ml, shaken for 2 min. Their absorption was then measured at the wavelength of 210 nm. This protocol was repeated on three consecutive days. The accuracy of the method was ultimately calculated based on statistical analysis results. Note that to have an acceptable accuracy for a method, the percentage of standard deviation should be less than 20%.

Preparing and measuring the sample absorption

The sample bowls were first washed with distilled water and completely dried using hot air oven with 30 °C and were filled up to 1 cm off the edge with the solutions of simulants, previously reaching 90 °C. To maintain the intended temperature of the simulants during contact with the tableware, the samples were put in an oven with the desired temperature. Once the contact time was finished, the simulants were withdrawn and maintained in glass tubes and refrigerator until measurement time.

In the next phase, to measure each sample, 3.5 ml of it was transferred to the test tube and 0.5 ml of uranin plus 1 ml of formaldehyde were added to it. After mixing, they were placed in a spectrophotometry device to measure their absorption.

In order to measure the absorption of the samples, the absorption of melamine-uranin-formaldehyde complex, formed from Mannich reaction, was first scanned within the range of UV (200–400 nm). Then, a wavelength at which the complex had the maximum absorption was specified. The device became zero with a solution, containing all available materials in the sample except for melamine. The absorption of the samples was measured at the wavelength with the maximum absorption of M-F-U complex.

Results

The results indicate that melamine standard solution has no absorption within the UV range (200–400 nm). Similarly did formaldehyde and uranin have no absorption spectrum within the UV range. However, when 0.5 ml of uranin was mixed with 1 ml of formaldehyde, it had an absorption at 205 nm (Figure 2). The scan of M-U-F complex, obtained from Mannich reaction within the UV range, indicated that the greatest absorption occurred at the wavelength of 210 nm (Figure 3). Accordingly, the wavelength of 210 nm was considered for monitoring the samples and measuring melamine quantitatively.

Calibration curve and validating the spectrophotometry method

The absorption of 10 solutions of M-U-F complex was measured with different concentrations of melamine (0.063–2.52 µg/ml) at the wavelength of 210 nm. The calibration curve was drawn using these results (Figure 4). Table 2 reports the results related to the method validation.

Measuring the migration rate of the samples

As stated previously, we had five types of melamine dish, two types of stimulant, and two times of 30 and 90 minutes for absorption to be measured individually in three iterations. The measured absorptions for each sample were incorporated in the formula of calibration line to determine the rate of melamine migrated from tableware to the simulants based on ppb. Tables 3 and 4 present the results as the mean of the three iterations for each sample. (Also see Tables 5 and 6)

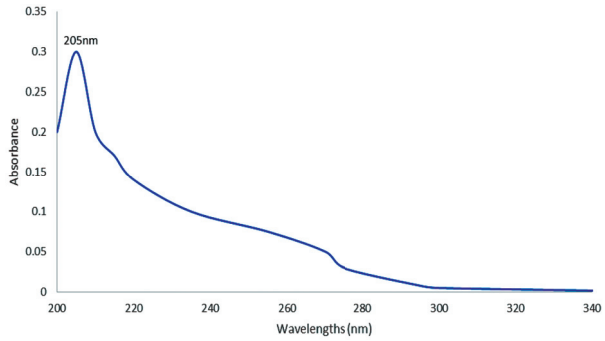

Figure 2. Scan of absorption spectrum of uranin-formaldehyde solution within the UV range

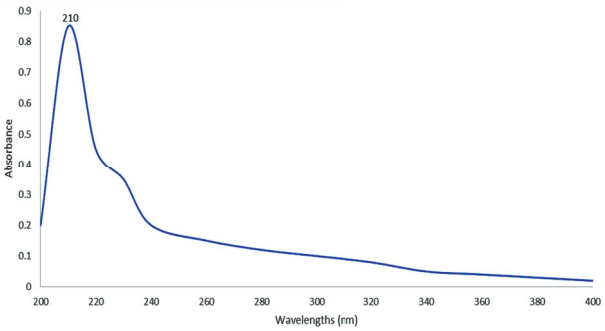

Figure 3. Scan of absorption spectrum of melamine-uranin-formaldehyde complex within the UV range

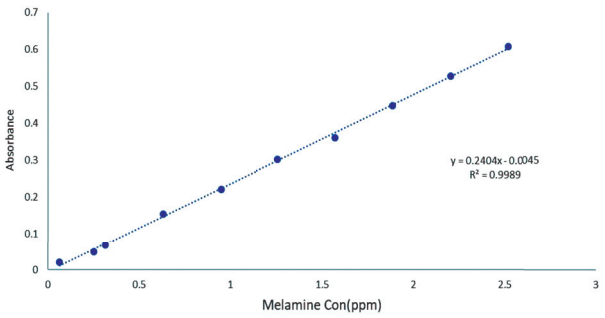

Figure 4. calibration curve of melamine-uranin- formaldehyde complex

Table 2. Results of validating the spectrophotometry method.

Parameter	Result
Maximum absorption	210 nm
Accuracy (Recovery%)	%95.3
Precision	96.2%
Slope	0.0002
Intercept	–0.0045
Linearity range (µg/ml)	0.063–2.520
Standard equation regression	y = 0. 240x – 0.0045
Correlation coefficient	R^2 = 0.9989
SE of intercept	0.00404
SD of intercept	0.01211
LOD (µg/ml)	0.19990
LOQ (µg/ml)	0.60578

Table 3. Results of migration rate of the samples through spectrophotometry method after 30 minutes of contact.

Mean ± SD (ppm)	Test	Sample
1.545+0.07	Water 90 °C - 30 min	Old dish
5.309+0.07	Acetic acid (3%) 90 °C - 30 min	
3.729+0.05	Water 90 °C - 30 min	Non standard dish
5.768+0.07	Acetic acid (3%) 90 °C - 30 min	
2.015+0.01	Water 90 °C- 30 min	Standard dish A
2.452 +0.02	Acetic acid (3%) 90 °C - 30 min	
2.127+0.01	Water 90 °C - 30 min	Standard dish B
3.111+0.009	Acetic acid (3%) 90 °C - 30 min	
2.625+0.02	Water 90 °C - 30 min	Standard dish C
5.956+ 0.01	Acetic acid (3%) 90 °C - 30 min	

Table 4. Results of migration rate of the samples through spectrophotometry method after 90 minutes of contact

Mean ± SD (ppm)	Test	Sample
1.678+0.06	Water 90 °C- 90 min	Old dish
7.604 +0.06	Acetic acid (3%) 90 °C- 90 min	
3.982+0.02	Water 90 °C- 90 min	Non standard dish
8.737+0.10	Acetic acid (3%) 90 °C- 90 min	
1.342+0.03	Water 90 °C– 90 min	Standard dish A
3.242+0.04	Acetic acid (3%) 90 °C- 90 min	
2.855+0.03	Water 90 °C- 90 min	Standard dish B
5.628+ 0.01	Acetic acid (3%) 90 °C- 90 min	
2.738+0.04	Water 90 °C- 90 min	Standard dish C
6.750+0.05	Acetic acid (3%) 90 °C- 90 min	

Discussion

In this study, a method was first designed and then validated using a spectrophotometry device for measuring the rate of melamine migration from tableware as there was no routine method of using spectrophotometry for checking the rate of melamine migration from food-related tableware and since the migration rate of melamine in food materials has not been examined in food quality control laboratories in Iran. The results indicated that this method is reliable with an acceptable accuracy and standard deviation. Given the acceptable values of limit of detection (LOD) and limit of quantitation (LOQ), this method can be functional for checking the rate of melamine migration from tableware to food. Further, statistical analysis of the results of measuring the melamine

Table 5. Summary of statistical comparisons of tableware types and simulants.

Simulant	Temperature	Tableware type (I)	Tableware type (J)	Mean Difference (I-J)	*p* value	95% Confidence Interval for Difference	
						Lower Bound	Upper Bound
Distilled water	90 °C	Old dish	Non standard dish	−0.868*	0.000	−1.086	−0.650
			Standard dish A	−0.022	1.000	−0.239	0.196
		Non standard dish	Old dish	0.868*	0.000	0.650	1.086
			Standard dish A	0.846*	0.000	0.629	1.064
		Standard dish A	Old dish	0.022	1.000	−0.196	0.239
			Non standard dish	−0.846*	0.000	−1.064	−0.629
Acetic acid (3%)	90 °C	Old dish	Non standard dish	−0.111	1.000	−0.329	0.107
			Standard dish A	0.813*	0.000	0.595	1.030
		Non standard dish	Old dish	0.111	1.000	−0.107	0.329
			Standard dish A	0.923*	0.000	0.706	1.141
		Standard dish A	Old dish	−0.813*	0.000	−1.030	−0.595
			Non standard dish	−0.923*	0.000	−1.141	−0.706

Table 6. Summary of statistical comparisons of tableware types, stimulants and temperatures.

Brand	Stimulant	Temperature (I)	Temperature (J)	Mean Difference (I-J)	*p* value	95% Confidence Interval for Difference	
						Lower Bound	Upper Bound
Old dish	Distilled water	30 °C	90 °C	−0.151*	0.040	−0.295	−0.007
		90 °C	30 °C	0.151*	0.040	0.007	0.295
	Acetic acid (3%)	30 °C	90 °C	−0.142	0.054	−0.286	0.002
		90 °C	30 °C	0.142	0.054	−0.002	0.286
Non standard dish	Distilled water	30 °C	90 °C	−0.181*	0.014	−0.325	−0.037
		90 °C	30 °C	0.181*	0.014	0.037	0.325
	Acetic acid (3%)	30 °C	90 °C	−0.235*	0.002	−0.379	−0.090
		90 °C	30 °C	0.235*	0.002	0.090	0.379
Standard dish A	Distilled water	30 °C	90 °C	0.030	0.686	−0.115	0.174
		90 °C	30 °C	−0.030	0.686	−0.174	0.115
	Acetic acid (3%)	30 °C	90 °C	−0.479*	0.000	−0.623	−0.334
		90 °C	30 °C	0.479*	0.000	0.334	0.623

migration rate, based on spectrophotometry, indicated that the designed model was significant (p<0.001).

Measuring the rate of melamine migration from melamine tableware to the simulants under different conditions by spectrophotometry showed that the migration occurred in all samples. However, the rate of migration in all samples was less than the standard level of the European Union for melamine migration, which is 30 µg/ml (SML).

The results also suggested that time was an important factor in melamine migration. It was observed that lengthening the contact duration of tableware with the simulants, when other variables were fixed, increased the migration significantly (p<0.05) in 93% of samples. This high percentage of the significant role of time is noticeable in raising melamine migration.

The other factor tested on melamine migration was the type of tableware in terms of being standard or non-standard as well as old and new, whose effects were measured on the rate of migration. It was seen that the type of tableware significantly influences the rate of melamine migration to the simulants (p<0.001). The statistical analysis of the results revealed old melamine tableware increased melamine migration in 41% of cases (p<0.05). Also, non-standard tableware affected the rate of migration such that in 90% of cases, being non-standard significantly (p<0.001) increased the rate of migration, and thus the effect of non-standard production of melamine tableware was more significant than the age of the tableware.

In a similar study, Rima *et al.* measured melamine migration through spectrophotometry method based on melamine-formaldehyde and a group of ketone complex. They managed to separate the melamine migrated to fish with a recovery (accuracy) percentage of 97% within similar results (Rima, 2009).

In another study, Rima *et al.* used the spectrofluorometric method to measure melamine migration.

Their method was based on Mannich reaction. With similar results to the current study, they could detect the melamine added to milk powder with a recovery (accuracy) percentage of 97% (Rima, 2009). Chansuvarn *et al.* also used spectrophotometry method in a similar study to measure contamination of milk and its products with melamine. Their method was also based on Mannich reaction. They stated that this is a reliable method for measuring melamine in food material samples (Chansuvarn, 2013).

Chik *et al.* measured melamine migration from 41 samples of food serving dishes to food simulants. In that study, samples were exposed to two types of food simulants (3% acetic acid and distilled water) under three test conditions (25, 70 and 100°C) for 30 min using LC-MS/MS device. The results of their study recommended that excessive heat and acidity may directly affect melamine migration from melamine-ware products (Chik, 2011)

Conclusion

Spectrophotometry method is a simple, cheap and available method for most food quality control laboratories. It is capable of measuring the acceptable accuracy of melamine and has the potential of being further researched and developed. Also, as melamine migration occurred across all samples though it was less than the standard level of the European Union in all of them, it should be noticed that long-term and continuous use of tableware, especially for long-term maintenance of hot food, may lead to bad effects, arising from toxicity with melamine. e, Developing methods for monitoring melamine in food materials is necessary to cope with its adverse effects, thus enhancing public health.

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