

Single-Run Electrochemical Determination of Melamine in Dairy Products and Pet Foods

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S Supporting Information

ABSTRACT: A simple electrochemical approach, which does not require any expensive and complex instruments, is established for the selective and quantitative recognition of melamine in dairy products and pet foods. During a preconcentration step (at 1.8 V versus Ag/AgCl), the formation of a polymer film from melamine on a preanodized screen-printed carbon electrode was identified by SEM and XPS. The as-formed polymer was found to be electroactive with a reversible redox peak, and hence square-wave voltammetry was applied to further increase the detection sensitivity to meet the detection limit for application in real sample analysis. Simply with a medium exchange procedure, melamine was selectively detected with a detection limit ($S/N = 3$) of $0.8 \mu\text{M}$ (i.e., 98.3 ppb) by square-wave voltammetry. Lower than 1 ppm of melamine in real samples can be easily detected with good recoveries of 98.7–100.9% by the proposed approach. The recovery tests established for external calibration and standard addition techniques verified that the analysis can be done in a single-run measurement.

KEYWORDS: melamine, infant milk formula, pet food, electrochemical, single-run detection

INTRODUCTION

Melamine is used primarily in the synthesis of melamine formaldehyde resins for manufacturing laminates, plastics, coatings, commercial filters, glues or adhesives, dishware, and kitchenware.^{1–8} It has been illegally added to dairy products to obtain high readings of total nitrogen content as the false measurement of protein level.⁹ Ingestion of melamine at levels above the safety limit (2.5 ppm in the United States and European Union; 1.0 ppm for infant formula milk powder in China) may cause kidney failure and even death.^{10,11} Melamine contamination was also found to be the cause for two outbreaks of food-associated renal failure in pets in 2004 and 2007.^{11,12} So far, monitoring melamine-tainted products continues to be a worldwide concern. There is always a need to establish a simple and highly sensitive assay method to solve this melamine detection problem in dairy products and pet foods.

Most techniques utilized for the determination of melamine require expensive and complicated instruments, making on-site, real-time melamine testing difficult.¹⁰ We present here a simple electrochemical approach for melamine analysis without dependence on expensive and complex instruments. Melamine is highly stable with poor electroactivity; hence, there are very few reports regarding the electroanalysis of melamine.¹³ Several indirect electrochemical methods were developed to improve the electroactivity of melamine.^{14–16} Our group also reported an electrochemical approach for sensitive detection of nonelectroactive melamine using a disposable screen-printed carbon electrode (SPCE) with uric acid as the recognition element.¹⁷ In this study, based on an idea adopted from the synthesis of melamine formaldehyde resins, we report an improved electrochemical method for melamine analysis.

Our previous studies have demonstrated that different oxygen functionalities on the surface of the SPCE can be easily manipulated under different preanodization treatments.^{18–23} We expect

that with the suitable formation of an oxo-surface group on the SPCE, for example, aldehyde in this occasion, melamine may react to form a polymer on the electrode surface. In other words, a simple medium-exchange procedure can lead to the selective recognition of melamine without any interference from other coexisting chemicals. With the help of this preconcentration step, highly selective determination of melamine in complicated real samples may thus be possible. Interestingly, the as-formed polymer was found to be electroactive with a reversible redox peak, and hence square-wave voltammetry (SWV) was applied to further increase the detection sensitivity to meet the detection limit for application in real sample analysis. This proposed electrochemical method was finally demonstrated for the determination of melamine in milk powder and pet food without the necessity of laborious sample preparation.

EXPERIMENTAL PROCEDURES

Chemicals and Reagents. Melamine (Sigma-Aldrich, USA) and all other reagents were of ACS-certified reagent grade and used without any further purification. A 0.1 M (pH 6.7) phosphate buffer solution (PBS) and all other aqueous solutions were prepared with Millipore (Billerica, MA) deionized water throughout this investigation. Famous brand milk powder (Anlene, New Zealand) and pet food (Pedigree, Thailand) samples were bought from a local supermarket. The tainted pet food sample was obtained from the official bureau of Taiwan. All real samples were treated by first dissolving 0.1 g of dried milk powder/pet food and 0.25 g of NaCl (Sigma, USA) in 10 mL of water. After 1 min of vortex shaking and 5 min of ultrasonication, the solution was heated until completely dissolved. Then the mixture was centrifuged at 4000 rpm for

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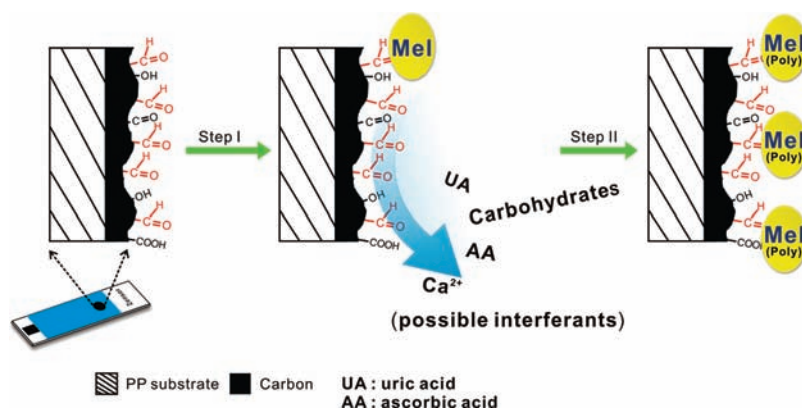


Figure 1. Schematic diagram of the proposed electrochemical method consisting of the preconcentration and medium-exchange steps.

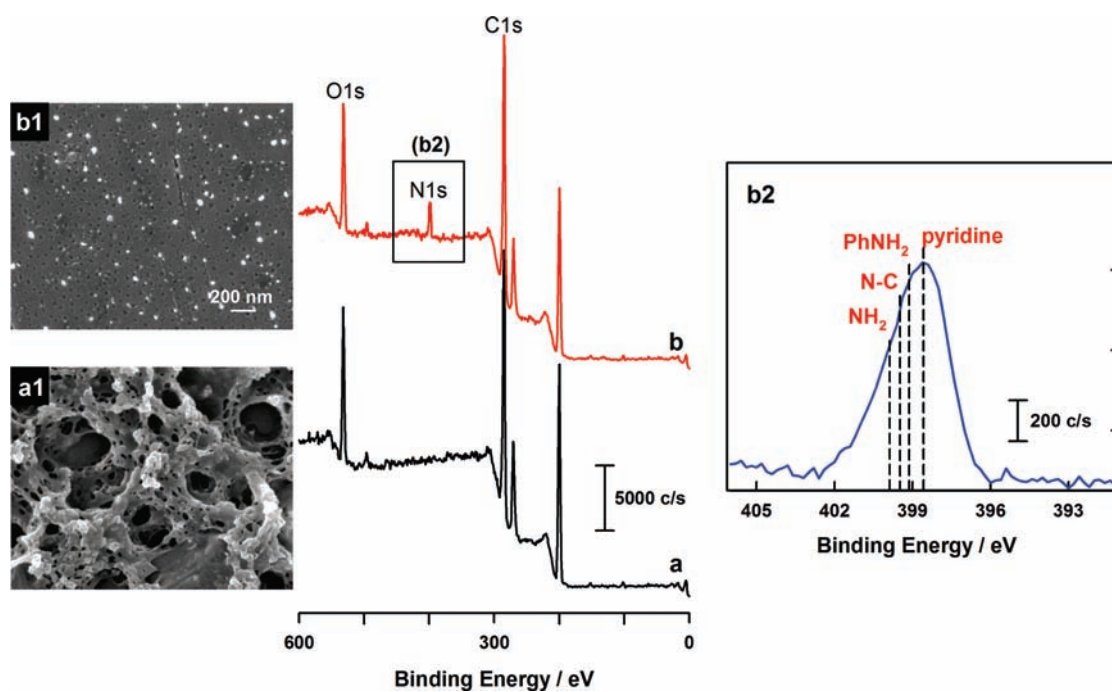


Figure 2. Typical SEM images and XPS spectra of (a) SPCE* and (b) SPCE*-Mel, respectively.

20 min and subsequently filtered through a 0.45 μm polytetrafluoroethylene membrane filter (Advantec, Japan). The sample solutions were suitably diluted for real sample analysis.

Apparatus and Electrodes. Electrochemical measurements were performed with a CHI832a electrochemical workstation (CH Instruments, Austin, TX) in a three-electrode cell assembly of a bare SPCE or the preanodized SPCE (SPCE*) working electrode, an Ag/AgCl, 3 M KCl reference electrode, and a platinum auxiliary electrode. The SPCE with a working area of 0.2 cm^2 and a conductive track radius of 2.5 mm was purchased from Zensor R&D (Taichung, Taiwan). X-ray photoelectron spectroscopy (XPS) analysis (Omicron DAR 400, Germany) was performed by using an Al $K\alpha$ X-ray source (1486.6 eV) with 0.1 eV resolution. The pressure inside the analyzer was maintained at about 10^{-10} Torr during the measurements. Prior to the experiments, the binding energy (BE) was standardized with Au4f_{3/2} (84.0 eV, full width at half-maximum (fwhm) = 1.20 eV). The C1s peak at 284.6 eV is taken uniformly as an internal standard. The high-resolution spectra were obtained under ambient conditions and averaged over a number of scans

to increase the signal-to-noise ratio. Quantitative XPS analysis was carried out by using a Sigma Plot 10.0 graphic program to pick up the intensity maximum and BE values. XPS peak areas were calculated by using built-in software programs. Peak-sensitive parameters were used to calculate the atomic ratio factors.

Procedure. Before further electrochemical experiments, the SPCE was equilibrated in 0.1 M (pH 6.7) PBS by cycling between -1.0 and 1.0 V for 20 segments. The SPCE* was prepared at an applied potential of 2.0 V for 300 s in 0.1 M (pH 6.7) PBS. Electroanalysis of melamine consists of two discrete steps as illustrated in Figure 1. In the first step, melamine is preconcentrated at the SPCE* under a deposition potential of 1.8 V versus Ag/AgCl for 300 s. Then, a medium-exchange procedure (step 2) is applied by submerging the preconcentrated SPCE* in pure water with stirring for 5 s to clean up the electrode. The as-prepared polymer film electrode was designated SPCE*-Mel. Then, SWV was applied for the selective recognition of melamine with optimized parameters of frequency = 15 Hz, amplitude = 25 mV, and step = 5 mV. All experiments were performed at room temperature (25 $^{\circ}\text{C}$).

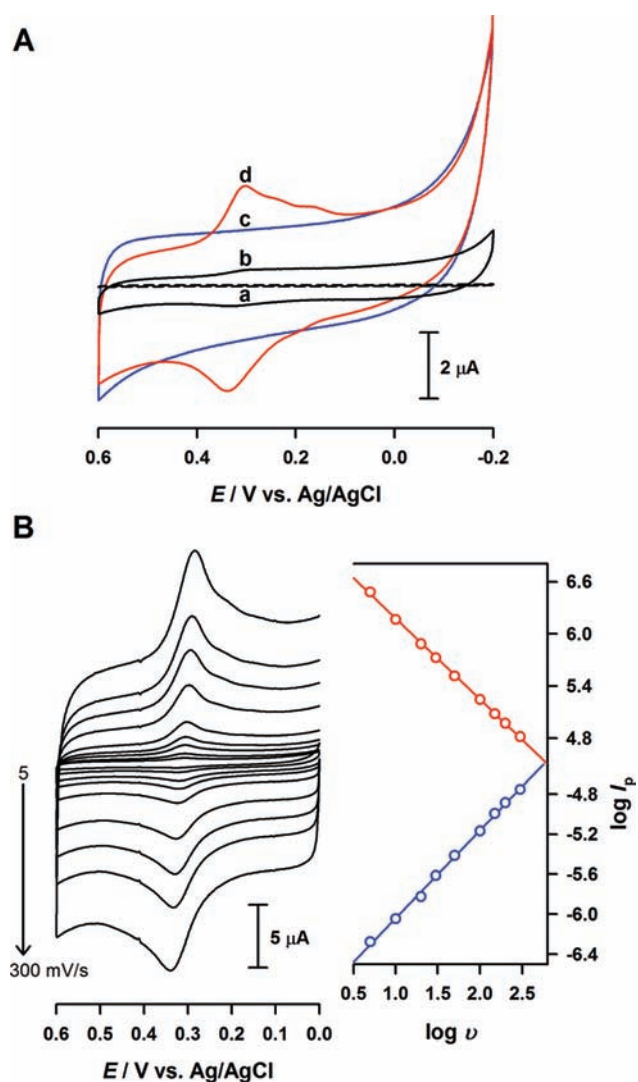


Figure 3. (A) Cyclic voltammograms in the absence/presence of melamine at a bare SPCE (a, c) and the SPCE* (b, d), respectively, at a scan rate of 50 mV/s. (B) Cyclic voltammetric responses and the obtained $\log(i_p)$ versus $\log(\nu)$ plots of the SPCE*-Mel in 0.1 M (pH 6.7) PBS under different scan rates.

RESULTS AND DISCUSSION

Formation of Polymer Film. To verify the formation of polymer film from melamine by the proposed electrochemical approach, the electrode surface was characterized by scanning electron microscopy (SEM) and X-ray photoelectron spectroscopy (XPS). As shown in Figure 2a1, the SEM picture of the SPCE* shows porous structure due to the surface reorientation to generate edge plane sites with surface carbonyl functionalities during preanodization.²¹ Compared to the highly porous surface of the SPCE*, a thin film was found to deposit on the electrode surface in the presence of melamine, as shown in Figure 2b1. Note that, for the same preconcentration time of 300 s, the film thickness was found to increase at higher concentration of melamine for the SPCE*-Mel (Supporting Information, Figure S-1).

XPS was further applied to study the surface characteristics of the polymer on the electrode surface. As illustrated in Figure 2b, the observation of typical N_{1s} XPS peaks confirms that the

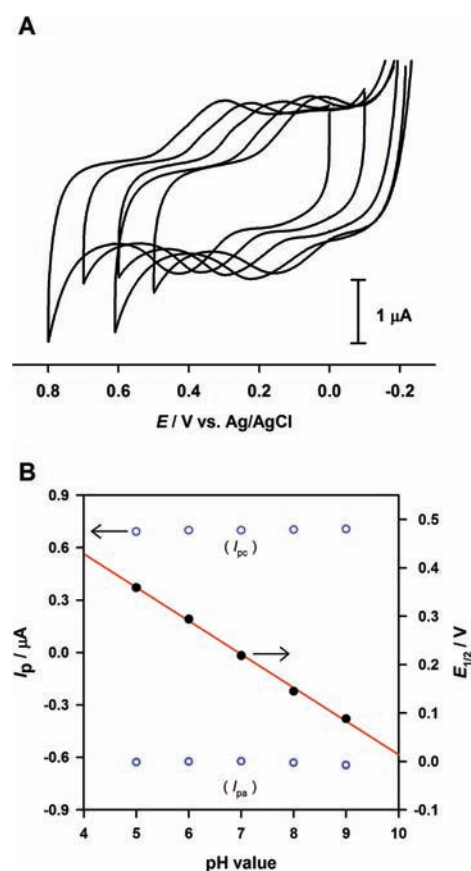


Figure 4. (A) Dependence of cyclic voltammograms on solution pH for the SPCE*-Mel at a scan rate of 20 mV/s. (B) Obtained peak currents and $E_{1/2}$ versus pH plots.

polymer film formation is related to melamine. Note that no such N_{1s} peaks were seen on the SPCE*. More detailed N_{1s} XPS levels, as shown in Figure 2b2, indicate the existence of pyridine (398.6 eV), PhNH_2 (399.1 and 399.2 eV), $\text{N}-\text{C}$ (399.5 eV), and $-\text{NH}_2$ (399.8 eV) groups; the formation of a melamine polymer film at the SPCE* can thus be proved from the above evidence.

Electrochemical Behavior. The electrochemical behavior of the as-formed melamine polymer film was next studied. Experiments were performed in the presence/absence of melamine at a bare SPCE and the SPCE* in 0.1 M (pH 6.7) PBS under a deposition potential of 1.8 V versus Ag/AgCl for 300 s. As can be seen in Figure 3A, in the absence of melamine, no peak responses were observed at a bare SPCE (curve a) and the SPCE* (curve c). On the other hand, the much higher background current for the SPCE* indicates the effective formation of an oxide layer during the preanodization process. In the presence of melamine, it is interesting that a pair of well-defined and highly reversible redox peaks was observed with a formal potential of ~ 0.32 V at both electrodes. The polymer formation obviously favors the surface condition at the SPCE* and hence with a much higher redox peak current at the SPCE* (curve d) by comparison to that at a bare SPCE (curve b). Most importantly, the electrochemical experiments again clearly indicate that the redox peaks are derived from melamine.

We conduct an easy experiment by studying the scan rate effect to the charge transfer process of the as-formed peak to verify the proposed approach to the selective recognition of

melamine and also to prove this polymer formation behavior. Figure 3B shows the cyclic voltammograms and the plot of peak current versus scan rate at different scan rates ranging from 5 to 300 mV/s. A good linear relationship for the peak current and scan rate undoubtedly indicates a surface-controlled electrode process due to the polymer formation at the SPCE*. The polymer formation process has a dramatic effect to achieve good selectivity for melamine with electrochemical activity at the SPCE* and can ensure the possibility of a simple and selective procedure for melamine determination in milk powder and pet food.

The electrochemical peak potential was found to be pH-dependent with an increase of the solution pH leading to a negative shift in potential for both anodic and cathodic peaks (Figure 4). The slope for a linear plot of $E_{1/2}$ versus pH is very close to the theoretical value of -59 mV/pH for a reversible, equal proton/electron redox reaction process. Because virtually the same peak current was observed for melamine in the pH range of 5–9, a single-run detection of melamine is thus possible using a built-in calibration plot, as will be discussed in the following section.

Analytical Performance. Although the proposed electrochemical approach is highly selective to melamine, to solve the

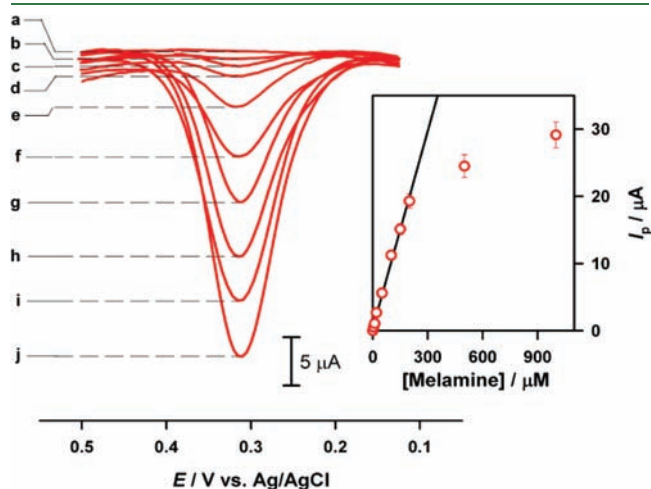


Figure 5. SWV responses of the SPCE*-Mel with increasing concentration of melamine in 0.1 M (pH 6.7) PBS. The concentrations of melamine were (a) 0, (b) 5, (c) 10, (d) 20, (e) 50, (f) 100, (g) 150, (h) 200, (i) 500, and (j) 1000 μM .

melamine detection problem in real samples, there is always a need to establish a highly sensitive assay method. It is well-known that the sensitivity of SWV is proportional to the degree of reversibility of the electrochemical reaction. Because the redox couple shows a reversible behavior, a clear advantage of using SWV with respect to the sensitivity of detection is expected. Under optimized SWV conditions, Figure 5A shows the typical SWV responses for melamine ranging from 5 to 200 μM in pH 6.7 PBS. A good linear calibration curve was obtained with a slope and regression coefficient of 99.3 $\mu\text{A}/\text{mM}$ and 0.996, respectively. The detection limit (signal/noise = 3) was calculated as 0.8 μM (i.e., 98.3 ppb) on the basis of the standard deviation observed for 10 successive detections of 5 μM melamine. Because the detection limit is much lower than the safety limits of 2.5 ppm (20 μM) and 1.0 ppm (8.0 μM) in the United States and China, respectively, the proposed method is satisfactory for application in real sample analysis.

Under the optimized conditions, the amounts of melamine in commercial milk powder and pet food were measured by the proposed electrochemical method. Table 1 summarizes the results of real sample analysis by standard addition method. Because the existing milk powder in the market is free of melamine, the sample was spiked with certain amounts of melamine standard solution directly. Excellent recoveries in the range of 98.7–100.9% were obtained for all samples. As to the tainted pet food, a value of 6179 mg/kg was detected by this method. This is close to the detected value of 6191 mg/kg using a commercial solid phase extraction coupled with HPLC-UV detection method.¹⁷ Note that the recoveries are much better than those achieved with a recently reported colorimetric method for melamine detection in dairy products, with recoveries up to 111% for raw milk and 115% for milk powder using a spectrophotometer.²⁴

These results demonstrate the high selectivity toward melamine, which is important whether milk powder or other foods are being investigated. Furthermore, due to the cloudy condition of pet food samples, interference is almost unavoidable in a spectroscopic method even with a tedious pretreatment. The electrochemical method is relatively insensitive to the cloudy condition and thus provides a possible way to monitor melamine without tedious sample pretreatment. Furthermore, this rapid method using a disposable electrode is readily suitable for quantification of melamine.

Table 1. Results for Real Sample Analysis^a

sample	dilution factor	original detected value (μM)	detected value (mg/kg)	spiked (μM)	found (μM)	recovery (%)
1 (tainted pet food)	500	98.07 \pm 2.80	6179	20	2.18 \pm 0.64	100.9
				50	49.46 \pm 1.71	98.9
2 (pet food)	100	na	na	20	19.93 \pm 0.71	99.7
				50	49.66 \pm 1.66	99.3
3 (milk powder)	100	na	na	50	49.46 \pm 1.73	98.9
				100	98.65 \pm 3.41	98.7

^a Average of four analyses.

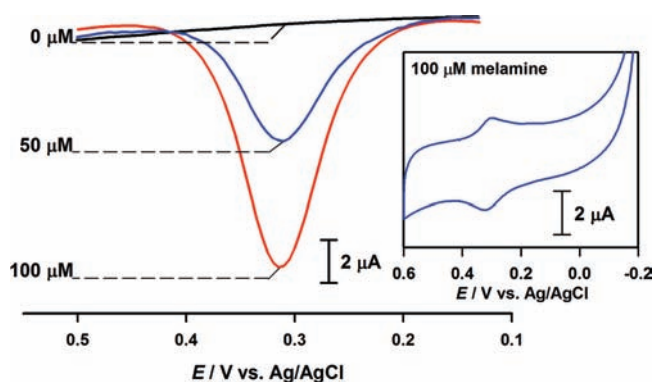


Figure 6. SWV responses of the milk powder dissolved in tap water spiked with 50 and 100 μM melamine. (Inset) Observed cyclic voltammogram after spike with 100 μM melamine.

The ultimate goal of this study is to develop a single test method with high sensitivity and good selectivity for the detection of melamine in real samples. A single-run approach with a disposable electrode and portable electrochemical instrument would benefit the field monitoring of melamine and result in a convenient method for analysis. Note that the standard addition method requires multiple measurements of samples. As mentioned earlier and demonstrated in Figure 4, virtually the same peak current was observed for melamine in the pH range of 5–9, making it possible to evaluate the single-run detection of melamine using a built-in calibration plot. Instead of using pH 6.7 PBS as in previous experiments, a commercial milk powder dissolved in tap water was used directly for real sample analysis. As shown in Figure 6, the pH value of the milk powder dissolved in tap water was measured as 6.8 according to the redox potential versus pH plot in Figure 4. Using the calibration plot from pH 6.7 PBS, good recoveries of 99.34 and 99.26% were observed for 50 and 100 μM spiked melamine, respectively. The results verify that the proposed single-run measurement of melamine is fast and reliable. The recovery tests established for external calibration and standard addition techniques verified that the analysis can be done in a single-run measurement.

We have successfully developed a simple electrochemical strategy for sensitive and selective detection of melamine. The proposed method relies on the formation of polymer from melamine at the preanodized screen-printed carbon electrode. The LOD (98.3 ppb) for melamine was significantly lower than the infant formula limit of 1 ppm in China. The results of good recoveries for melamine residue determination in milk powder and pet food are acceptable under world regulations. A single-run approach with the combination of disposable screen-printed carbon electrode and portable electrochemical instrument is actually suitable for on-site, real-time melamine testing.

■ ASSOCIATED CONTENT

Supporting Information. Additional information as noted in text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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