Supplementary material (ESI) for Chemical Communications

Electronic Supporting Information

for

Visual and light scattering spectrometric detections of melamine with polythymine-stabilized gold nanoparticles through specific triple hydrogen-bonding recognition

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Experimental Details

Apparatus

Localized surface plasmon resonance light scattering (LSPR-LS) signals and extinction spectra were measured with F-4500 fluorescence spectrophotometer (Hitachi Ltd., Tokyo, Japan) and UV-Vis-NIR spectrophotometer (Shimadzu, Tokyo, Japan), respectively. The LSPR-LS spectra measurements were made according to former reports [1-5] by simultaneously scanning the excitation and emission monochromators of the F-4500 spectrofluorometer with same starting excitation and emission wavelengths, during which the spectral bandwidths of both excitation and emission monochromators were kept at 5.0 nm. Scanning electron microscopic (SEM) measurements were performed on an S-4800 electron microscope (Hitachi Ltd., Tokyo, Japan). A N5PCS submicron particles size analyzer (Beckman coulter, Miami, USA) was used to detect the size of the aggregation species in solution on the basis of dynamic light scattering (DLS) principle. Nuclear magnetic resonance (NMR) spectra were recorded on AVANCE AV-300 (BRUKER, Swiss) with the reference of ^{1}H NMR chemical shifts of the residual solvent peak at 2.50 ppm in DMSO-*d*6. High

performance liquid chromatographic (HPLC) detection was performed with the equipments of LC2010A/C (Shimadzu, Tokyo, Japan) coupled with a Shim-pack VP-ODS 150 × 4.6 column, a CBM-20Alite system controller, two LC-20AT pumps, a CTO-10ASvp oven, and a SPD-20AV detector.

Materials

Melamine, commercially purchased from J&K Chemical Ltd (Beijing, China), was firstly dissolved with 5.0 mL methanol and then diluted with water. Oligonucleotide sequences, including polyA$_9$, polyC$_9$, polyG$_6$, polyT$_9$, polyT$_{12}$, polyT$_{23}$, polyT$_{33}$ and polyT$_{55}$, were purchased from Shengong Genetech Co. Ltd (Shanghai, China) and directly dissolved in water. Phosphate buffer (0.2 M, pH 7.6) was used to control the acidity according to the literatures.[6, 10] All reagents were of analytical reagent grade without further purification. Water used throughout was ultra-purified with LD-50G-E Ultra-Pure Water System (Lidi Modern Waters Equipments Co. Ltd, Chongqing, China) to keep the conductance not less than 18.0 MΩ. In the ^{1}H NMR spectroscopic measurements, melamine and thymine should be dissolved at first in dimethylsulfoxide (DMSO), and then some water could be allowed to add in for their poor solubility in water.

Preparation of AuNPs

AuNPs were prepared by reducing HAuCl$_4$ with citrate sodium following the literatures,[11-12] wherein citrate sodium serves as both reductant and stabilizer. Briefly, 50 mL of HAuCl$_4$ solution with final concentration of 10 nM was firstly prepared and heated to boiling, during which vigorously stirring should be made. 1.0 mL of 5% trisodium citrate was added to the solution quickly just as the solution began to boil. The color of the mixture changed from pale yellow to deep red within 3 min. By keeping boiling for another 5 min, the solution was cooled to room temperature (about 27 ^{o}C) and transferred for the UV–vis absorption and scanning electron microscopic measurements, and then stored in 4 ^{o}C refrigerator. The prepared AuNPs have the size of about 13 nm.

General Procedures

100 μL of 10 nM AuNPs was firstly added into a 1.5-mL plastic tube, and 100 μL of 0.23 μM polyT_{55} was then pipetted into the solution. Vortex-mixed and stood for 5 min at room temperature (about 27 ^{o}C). Appropriate water, melamine, and 50 μL of 0.2 μM pH 7.6 phosphate buffer were added into the mixture. The mixture was then vortex-mixed thoroughly, and kept at room temperature for another 5 min. After that, the mixture was transferred for LSPR-LS, absorption, SEM, and dynamic light scattering (DLS) measurements. It should be noted that the SEM imaging was made only after drying 5 μL of above solution dropped onto the aluminum foil.

Preparation of Milk Samples

Briefly, 2 mg of milk power or 2 mL raw milk was first mixed with water and the total volume was kept 50 mL. The milk suspension was then diluted 10 times with water, ultra-filtrated with a 10 KD ultrafiltration membrane, and centrifugated at 20000 rpm/min for 30 min. The ultra-filtrated milk samples were further 10000-fold diluted. After that, 30 μL 1.0×10^{-5} M melamine (37.8 ng) were artificially added in to 1.0 mL of above diluted milk samples for LSPR-LS or HPLC detections. For both PRLS and HPLC detections, three parallel samples were detected.

HPLC Detections

In order to identify the detection results of the proposed LSPR-LS method for melamine in real samples such as milk products, HPLC detections were made for comparison following the accepted standard method. [13] Ion-pairing buffer was employed, which consists of 2.10 g/L citric acid and 2.16 g/L sodium octanesulfonate. This ion-pairing buffer was adjusted to pH at 3.0 with NaOH solution. The absorption at the wavelength of 235 nm and 240 nm were monitored simultaneously and the data at 235 nm was recorded for chromatogram. The column temperature was kept at 40 ^{o}C, and a mobile phase of 88:12 buffer:methanol, which was filtered prior to use with a Millipore HPLC solvent filtration system and 0.45-μm membrane filters, was delivered at a flow rate of 1.0 mL/min.

Figures

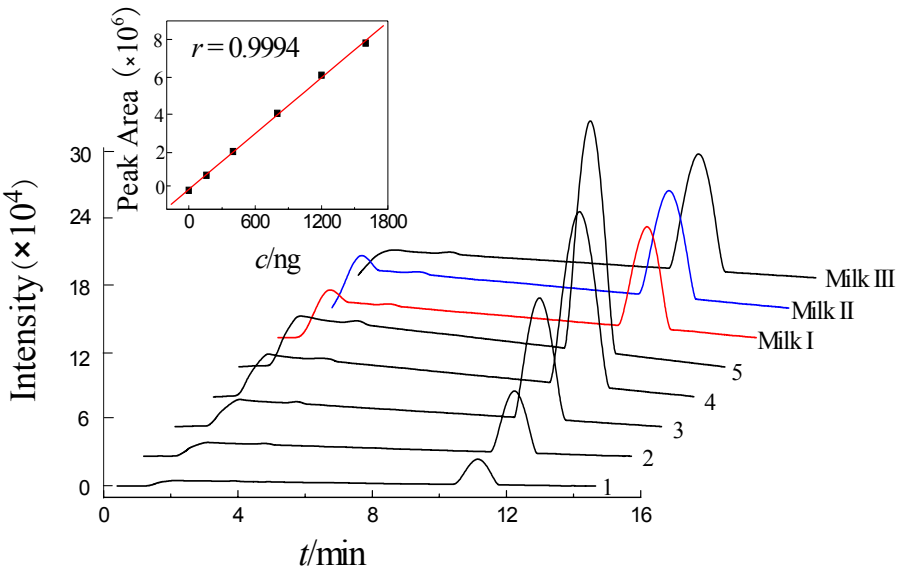
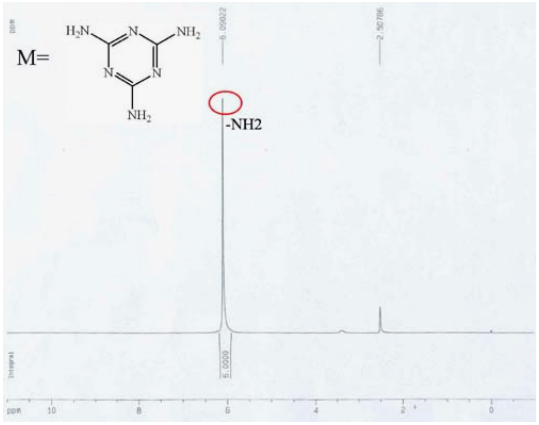
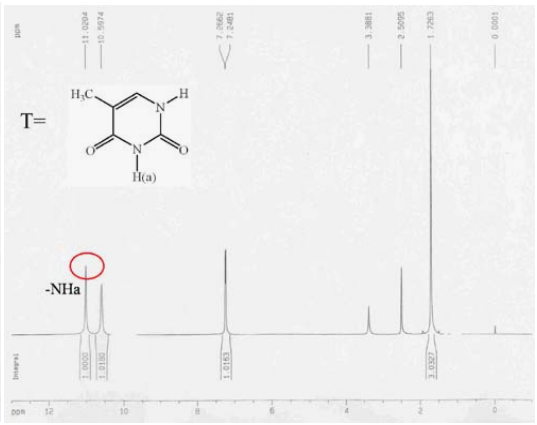


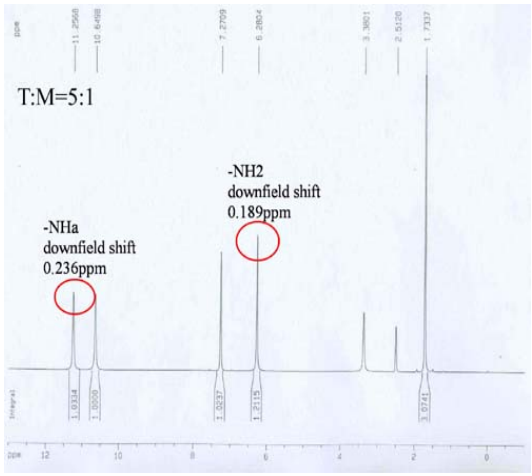
Fig. S1 HPLC analysis for the milk sample. Black curves are used to construct calibration as displayed in the inserted picture, which could be expressed as P_A= 4940.5 m + 53642, wherein P_A is the peak area and m is the amount of melamine. Melamine (Curves 1-5, ng), 160, 400, 800, 1200, 1600.

Fig. S2 The detailed in the ^{1}H NMR spectra in DMSO medium (A) and in 9:1 DMSO: H_2O medium (B)

Spectra Result A. ^{1}H NMR spectra in DMSO medium


M=
-NH2


T=
-NHa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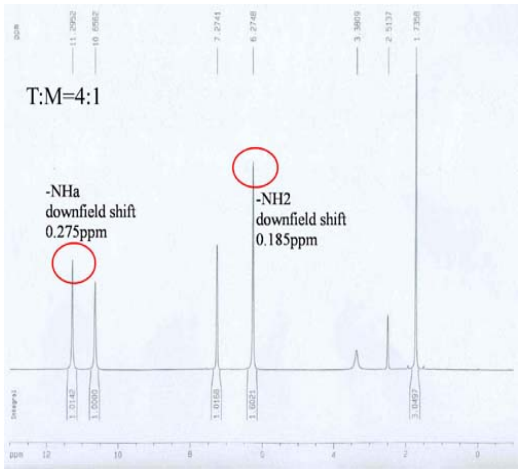
downfield shift
0.236ppm
-NH2
downfield shift
0.189ppm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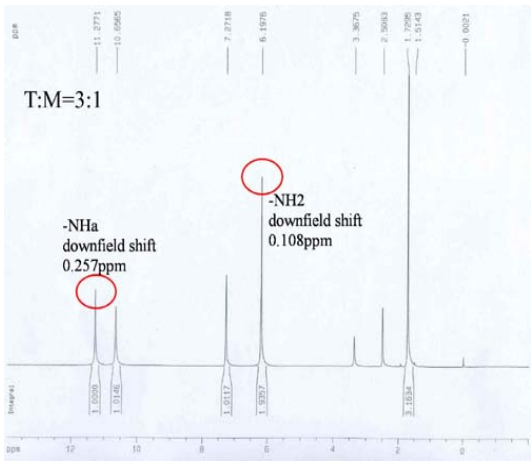
downfield shift
0.275ppm
-NH2
downfield shift
0.185ppm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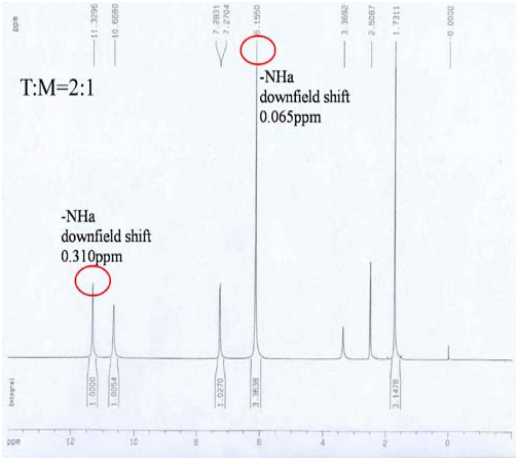
downfield shift
0.257ppm
-NH2
downfield shift
0.108ppm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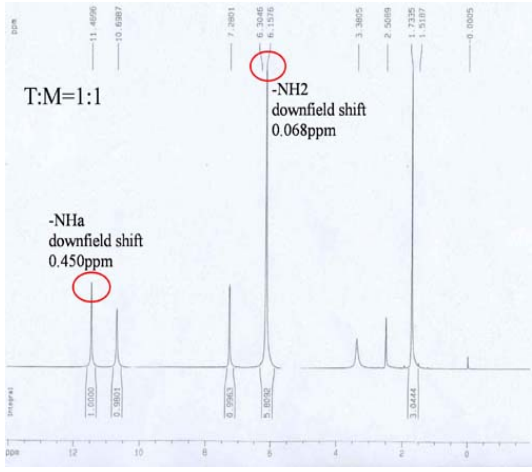
0.310ppm
-NHa
downfield shift
0.065ppm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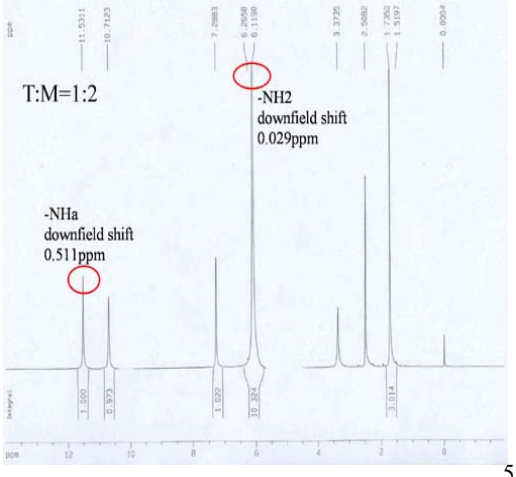
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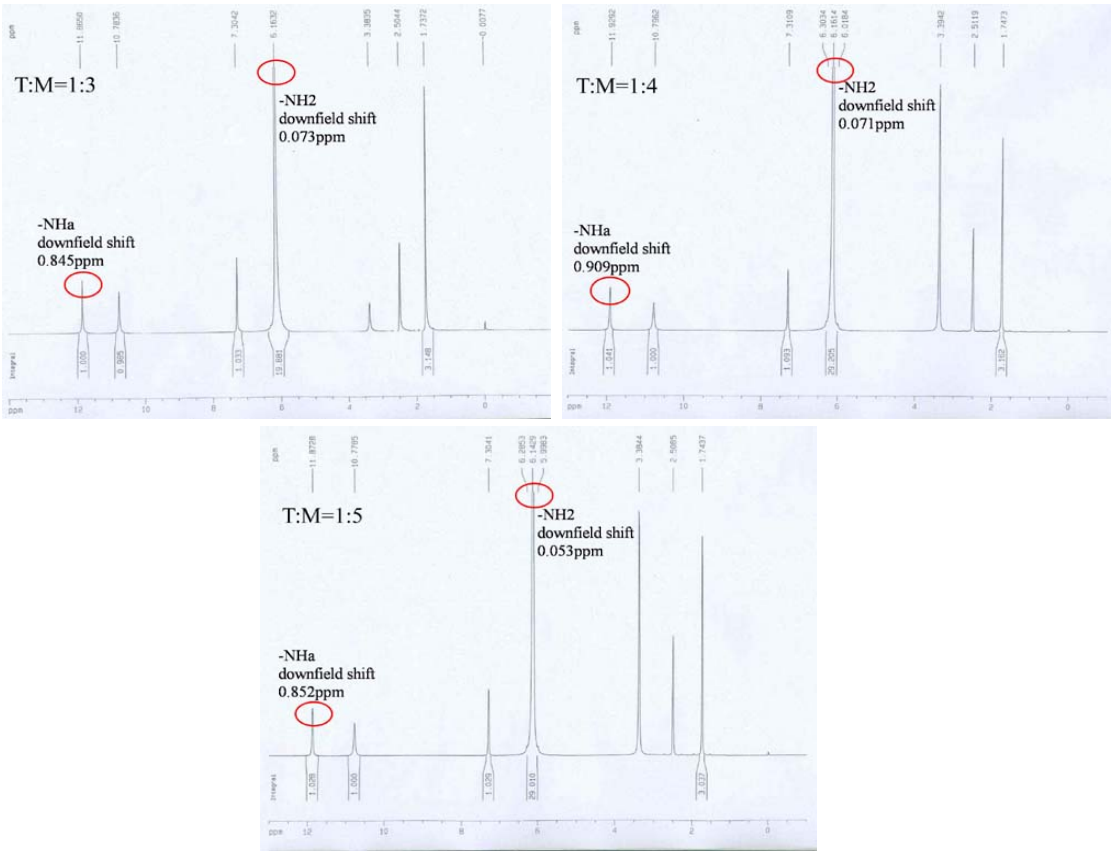
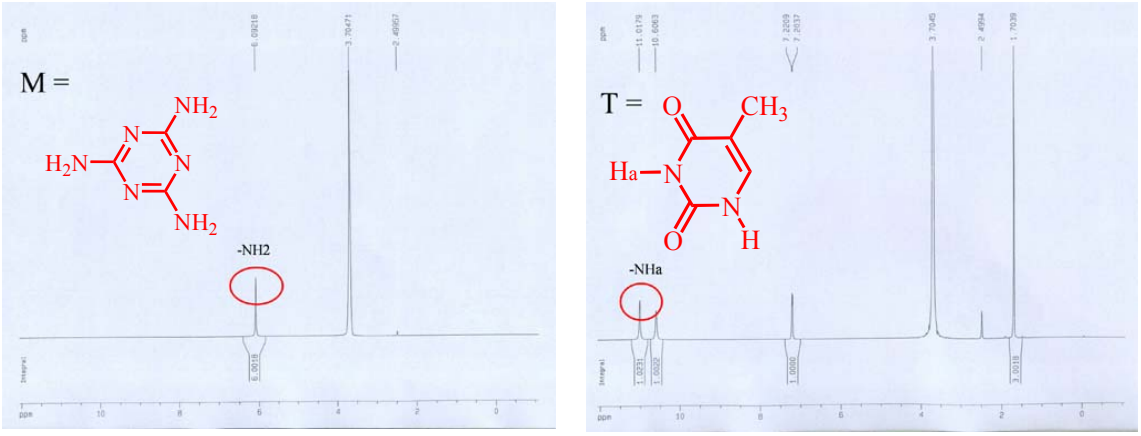
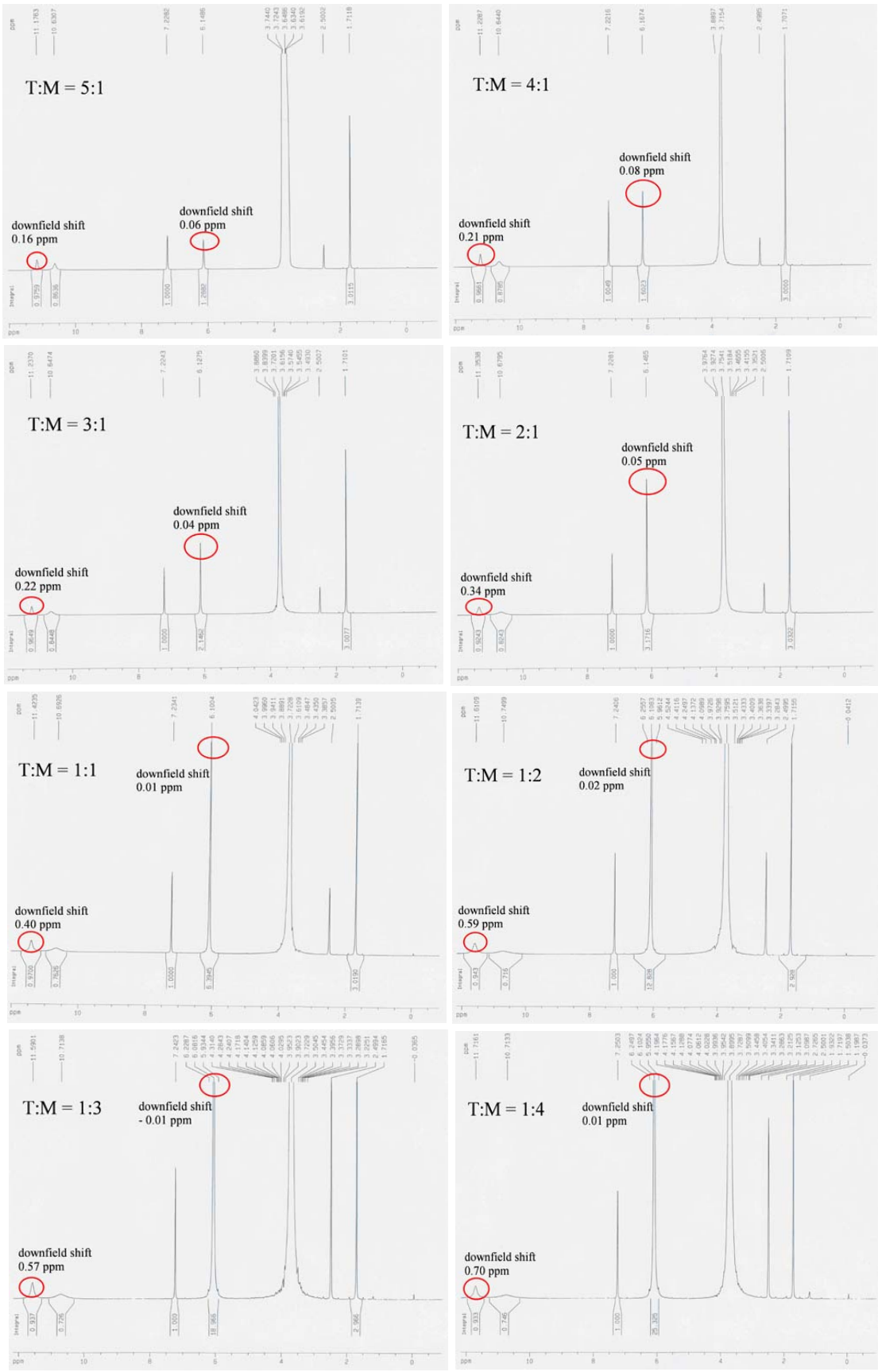


Spectra Result B. ^{1}H NMR spectra in 9:1 DMSO: H_2O medium.




T:M = 5:1
downfield shift
0.16 ppm
downfield shift
0.06 ppm
T:M = 4:1
downfield shift
0.08 ppm
downfield shift
0.21 ppm
T:M = 3:1
downfield shift
0.04 ppm
downfield shift
0.22 ppm
T:M = 2:1
downfield shift
0.05 ppm
downfield shift
0.34 ppm
T:M = 1:1
downfield shift
0.01 ppm
downfield shift
0.40 ppm
T:M = 1:2
downfield shift
0.02 ppm
downfield shift
0.59 ppm
T:M = 1:3
downfield shift
- 0.01 ppm
downfield shift
0.57 ppm
T:M = 1:4
downfield shift
0.01 ppm
downfield shift
0.70 ppm

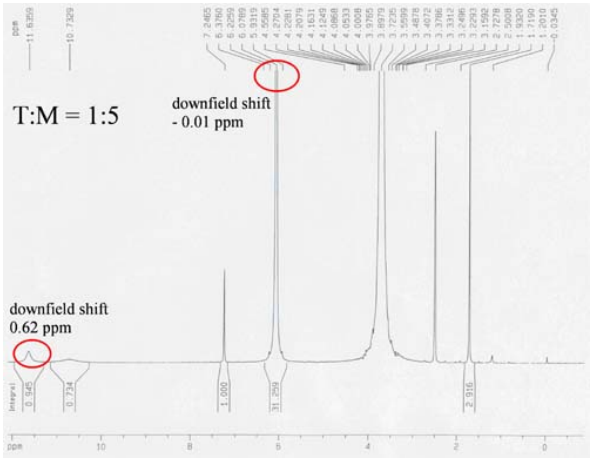


Table S1 Determination results of melamine in the milk samples by the proposed method and HPLC.

Sample	Proposed Method	HPLC
	Mean [a] ± SD [b](*n*=3, ng/mL)	Mean [a] ± SD [b](*n*=3, ng/mL)
Milk I	41.0 ± 2.0	37.5 ± 0.2
Milk II	37.4 ± 2.3	39.5 ± 0.3
Milk III	39.0 ± 2.6	39.9 ± 0.1

Three milk samples were filtrated through 10 KD ultrafiltration membrane at first, 10000-fold diluted, and then 30 μL 1.0×10^{-5} M melamine (37.8 ng) was artificially added in to 1.0 mL of the diluted milk samples. All the experiment condition of both HPLC method and proposed method is kept the same as that in Figure 6 and Figure 7.
[a] Mean of three determinations (*n*=3). [b] SD, standard deviation.

Table S2 Specificity test.

Substance	Concn. coexisting ($\times 10^{-5}$ M)	Change of LSPR-LS intensity (%)	Substance	Concn. coexisting ($\times 10^{-5}$ M)	Change of LSPR-LS intensity (%)
Na(I),Cl^-	8	4.8	Vc	1	-5.7
K(I),Cl^-	8	-6.2	VB_2	0.1	7.2
Mg(II),Cl^-	8	6.2	VB_{12}	1*	-6.6
Cu(II),Cl^-	8	9.6	BSA	1*	-1.6
Na(III),PO_4^{3-}	8	2.3	HSA	1*	-9.0
Al(III),SO_4^{2-}	4	-6.1	Glucose	100*	7.5
Ca(II),Cl^-	4	3.7	Lactose	100*	2.2
Zn(II),SO_4^{2-}	4	8.1	Maltose	100*	-9.2
Fe(III),Cl^-	4	5.4	Saccharose	100*	0.3

* g/mL. All the values obtained in the table were obtained according to the standard procedure, and concentration of reagents is as follows: AuNPs, 2 nM; poly-T_{55}, 0.046 □M; melamine, 800 nM; pH 7.6.

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